JOURNAL OF DAIRY SCIENCE

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Vol. XXIV, No. 7, July, 1941

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Published at
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The Journal of Dairy Science is issued monthly. Subscription is by the volume and one volume is issued per year.

Manuscripts should be typewritten and carefully revised before submission to T. S. Sutton, The Ohio State University, Columbus, Ohio. Twenty-five reprints will be furnished gratis to authors. Cost of additional reprints and reprint order blank will be submitted with proof.

The use of material published in the Journal is encouraged and a liberal policy will be followed concerning reproduction of articles with proper notation as to source.

Subscriptions. Price; $6.00 per volume in United States and Canada; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Correspondence regarding business policies of the Journal should be addressed to the Secretary-treasurer, R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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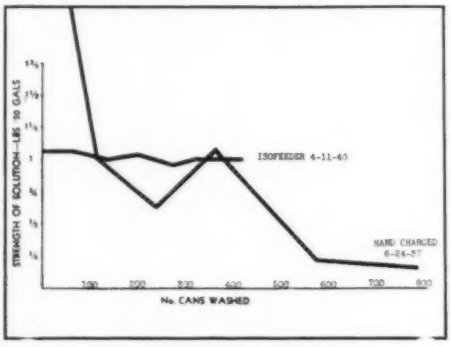

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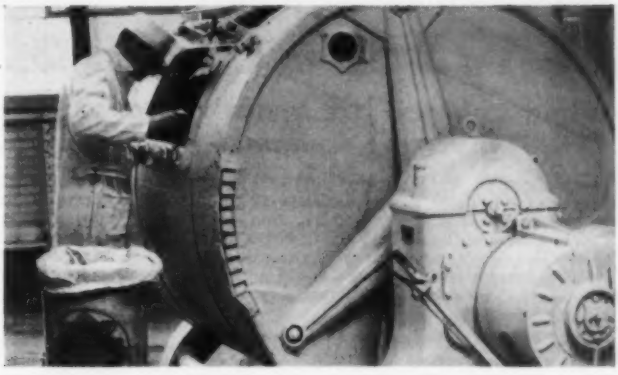

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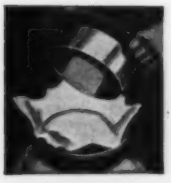

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIV JULY, 1941 NUMBER 7

THE FEEDING VALUE OF GRASS SILAGE IN THE RATION FOR DAIRY COWS

O. L. LEPARD[1] AND E. S. SAVAGE

Department of Animal Husbandry, Cornell University, Ithaca, New York

The ensiling of legumes and grasses has been practiced to a limited extent for years, although the practice has not been generally accepted because of recurrent failures. Recent investigations have shown possible methods of preventing these failures. The ensiling of legumes and grasses by these methods has been spurred on during recent years by extension workers, interested in the production of better quality roughage, and by commercial companies, interested in the sale of preservatives or equipment used in the process of ensiling.

The terminology with reference to ensiled crops is not uniform. Throughout this report the terminology of Bender and Savage (2) will be used. They used the term "grass silage" as an all-inclusive term referring to any "silage made from an uncured hay crop—whether it be a true grass such as timothy, a legume such as alfalfa, or a green cereal such as oats. Terms like 'alfalfa-molasses silage' or 'timothy-phosphoric acid silage' describe specific kinds of grass silage."

Among the feeding experiments with grass silage, Watson and Ferguson (15) have made extensive reports. They fed respective groups of dairy cows A.I.V. silage, molasses silage, and artificially dried grass. A statistical analysis of the milk production, butterfat production, and live weight changes showed no significant difference when equal amounts of starch equivalent and digestible crude protein were furnished in each ration.

A comparison of alfalfa silages prepared by the A.I.V. and molasses methods was made by Bohstedt *et al.* (3) in which feeding trials were carried out for three years on milking animals. During the three years the milk and butterfat production records were maintained almost equally well, although the molasses silage had a slight advantage. Each animal received 1 oz. of $CaCO_3$–Na_2CO_3 mixture (10 : 3) per 15 pounds of silage.

Bender *et al.* (1) have shown that molasses grass silage will replace corn

Received for publication January 25, 1941.

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silage or hay in the ration of a dairy cow without influencing the production level of the cows to a marked extent. When fed in place of both corn silage and hay, the production level was maintained, although there was a slight loss in body weight. It was believed that a slight increase in the grain ration could have overcome the slight loss in body weight manifested by the animals fed grass silage as the only roughage.

Alfalfa hay grown on irrigated land was compared with mixed grass and clover hay and grass silage by Hodgson and Knott (6). They fed a concentrate mixture according to production. The cows on the mixed hay and silage ration consumed 78.6 per cent as much dry matter as the alfalfa hay group. The cows fed alfalfa hay lost only 33 per cent as much live weight as the cows receiving the mixed hay and grass silage ration. The production of milk for the cows on the mixed hay and grass silage ration was 94.2 per cent as much as for those on the alfalfa ration.

Hayden *et al.* (5) fed two groups of milking cows A.I.V. alfalfa silage and alfalfa hay respectively. By a reversal method they found no significant difference in the production of the animals in the two groups. The A.I.V. silage was supplemented with four ounces of ground limestone per cow per day.

Many other feeding experiments (8, 11, 12, 13, 14) have been conducted to determine the relative value of different silages, different plants in dry or ensiled form, and different combinations of these plants. These experiments were usually of short time duration and, almost without exception, showed no significant difference between the various feeds when they were of good quality and the dry matter intake of the animal is constant.

The work here reported from the Cornell University Experiment Station covers four feeding experiments with milking animals. These experiments were designed to compare grass silage preserved with phosphoric acid with grass silage preserved with molasses, and to determine their value in replacing varying amounts of corn silage and dry hay.

The general procedure for all experiments included complete feed and production records. The animals were milked and fed twice daily. The gain or loss of body weight was determined from the average of three daily weights of the cows, taken at the beginning and end of each period in all experiments.

The chemical analyses of all feeds and the digestion coefficients used in calculations are given in tables 4 and 5. The analysis of the grain mixture was computed from Morrison's tables (9). This computed analysis was used in determining the consumption of feed nutrients in the grain mixture.

EXPERIMENT I

In this experiment the basic ration of one pound of dry hay and three pounds of corn silage per hundred pounds of body weight was varied by the

replacement of a part of the corn silage or dry hay, or both, with grass silage, and the effect on production and body weight determined. A triple reversal experiment was planned with five milking animals in each of three groups, A, B, C. The groups were balanced as closely as possible in milk production, fat test, body weight, breed, age, date bred, and condition.

The three rations used were as follows:

Ration I:

Corn silage—3 pounds for each 100 pounds live weight.
Mixed hay—1 pound for each 100 pounds live weight.
Grain—an 18 per cent concentrate mixture according to production.

Ration II:

Corn silage—3 pounds for each 100 pounds live weight.
Mixed hay—⅓ pound for each 100 pounds live weight.
Molasses silage (grass, clover and alfalfa)—2 pounds (equivalent to ⅔ pound of mixed hay) for each 100 pounds live weight.
Grain—an 18 per cent concentrate mixture according to production.

Ration III:

Corn silage—2 pounds for each 100 pounds live weight.
Mixed hay—⅔ pound for each 100 pounds live weight.
Molasses silage (grass, clover and alfalfa)—2 pounds (equivalent to ⅔ pound mixed hay) for each 100 pounds of live weight.
Grain—an 18 per cent concentrate mixture according to production.

The corn silage was well-eared and at a medium stage of maturity.

The grass silage was preserved with 41.75 pounds of blackstrap feeding molasses per ton of green crop. The approximate per cent of each crop in the silage was as follows: grasses—40 per cent; clovers—30 per cent; alfalfa—20 per cent; weeds—10 per cent. The crop was cut early and stored in the silo with a minimum of drying. After the usual spoilage at the top, the rest of the silage came out in excellent condition.

The dry hay was mixed grasses, clovers, and alfalfa of good quality, green and early cut. It was unofficially graded as U. S. No. 1 mixed hay. It was selected as being comparable to a type of hay that could have been made from the green crop used for grass silage under desirable weather conditions.

The grain used was a regular 18 per cent protein commercial open formula mixed feed as follows:

480 lbs. corn gluten feed
200 " wheat bran
100 " hominy feed
220 " ground oats
220 " coconut oil meal
300 " corn distillers dried grains

100 " soybean oil meal (hydraulic or expeller process)
160 " ground barley
180 " cane molasses
20 " steamed bone meal
20 " common salt

The three rations were fed to the respective groups in the following sequence:

	Ration I	*Ration II*	*Ration III*
First 5-week period	Group A	Group B	Group C
Second 5-week period	Group C	Group A	Group B
Third 5-week period	Group B	Group C	Group A

In determining results the feed consumed and milk produced were totalled from the three groups on each ration and reduced to the basis of one cow for one day. These final data are given in table 1.

This table indicates that the production of the animals on the different types of rations was practically the same. The greatest difference in daily average production of 4 per cent fat-corrected milk was 1.2 pounds. This is not a significant difference. The animals on Ration I had a slight advantage in that they received more total digestible nutrients. This caused a gain in weight of the animals on this ration.

EXPERIMENT II

The effect of continuous feeding of molasses grass silage as the only roughage was studied. Ordinary dairy practices call for both a succulent and a dry feed, and it is usually supposed that a combination of feeds is advantageous over a single feed.

A continuous 12-week feeding experiment was planned. Eleven animals were selected which were in fairly heavy milk production, but had passed their peak of lactation. All animals were less than three months pregnant at the end of the experiment. All of the animals were in good and thrifty growing condition. Animals of various breeds and body weights were used in the experiment.

The maximum amount of molasses silage (grass, clover and alfalfa) that the animals would consume was fed. The body weights and the condition of the animals were very closely watched and enough grain was fed to maintain both normal milk production and body weight. It was assumed that the animals' milk production should not decline faster than a normal production curve, and that they should gain weight slightly during advancing lactation, or at least maintain weight during the period of this experiment.

Molasses silage (grass, clover and alfalfa) was fed from the same silo as in Experiment I. Each animal was fed as much of this silage as it would consume. The grain was of the same mix as that used in Experiment I. An attempt was made to feed it in such quantities that the milk production and body weights be maintained in a normal way.

TABLE 1

Daily average feed consumption, nutrient consumption, weight change, and production of animals on the various rations of Experiment I, II, III, and IV

Ration	Grain	Dry hay, lb.	Molasses grass silage, lb.	Phosphoric grass silage, lb.	Corn silage, lb.	T.D.N. fed, lb.*	Digestible protein fed, lb.*	Milk produced, lb.	Butterfat percentage	Fat produced, lb.	Production 4% fat-corrected milk, lb.†	T.D.N. required for maintenance and production‡	Digestible protein required, lb.‡	Excess T.D.N.	Excess lb. digestible protein	Weight change, lb.
Daily average per cow for Experiment I (3 periods)																
I	7.7	11.2	.....	.....	33.4	16.4	2.22	26.5	3.66	.97	25.1	16.9	1.95	− .5	+ .27	+ .96
II	7.6	3.7	21.1	.....	33.1	14.9	2.06	26.7	3.70	.99	25.5	17.1	1.97	− 2.2	+ .09	+ .25
III	7.9	7.3	20.9	.....	22.2	15.6	2.20	28.0	3.58	1.00	26.3	17.3	2.01	− 1.7	+ .19	+ .39
Daily average per cow for Experiment II																
	16.44	.....	71.77	.....	.....	21.33	3.60	43.04	3.83	1.65	41.96	22.82	2.81	− 1.49	+ .79	+ .03
Daily average per cow for Experiment III (2 periods)																
I	10.2	.....	28.7	.....	33.9	19.7	3.1	28.8	4.26	1.23	29.93	18.4	2.2	+ 1.4	+ .9	+ .04
II	10.2	.....	.....	32.9	32.3	19.4	2.5	27.9	4.33	1.21	29.32	18.2	2.1	+ 1.2	+ .4	+ .06
Daily average per cow for Experiment IV (2 periods)																
I	13.87	10.28	.....	37.32	.....	22.6	3.7	36.9	4.13	1.52	37.58	20.4	2.5	+ 2.3	+ 1.1	− .37
II	13.78	9.81	29.49	.....	.....	21.9	4.1	36.6	4.09	1.49	37.06	20.7	2.5	+ 1.2	+ 1.6	− .41

* Calculated from average of analyses made during experiment (tables 4 and 5).
† Calculated according to Gaines (4).
‡ Calculated according to recommended standards by Morrison (9) for each animal and totalled.
Weight used was average of initial and final weights for period.

Table 1 gives records of the feed consumed, nutrients consumed, weight change, and production of the animals on Experiment II. This shows clearly that a very constant weight was maintained. This, however, was accomplished with a rather high rate of grain feeding. This high rate of grain feeding was necessary in order to make up for the lack of total digestible nutrients consumed in the roughage part of the ration.

Because this was a continuous feeding experiment with no control group, the production of these animals must be compared with normal.

Landis (7), working with Savage, plotted 389 lactations of animals from three large herds. These animals gave, on the average, a 2 per cent decline per week between the peak of lactation and the fifth month following breeding. Because these records were largely taken from experiment station herds, the normals derived should be comparable to those used in the experiment.

Table 2 gives the average daily production for each week of the 11 animals, and their calculated production (with a normal 2 per cent per week decline) according to Landis and Savage. It is indicated clearly in table 2 that the production of animals on the continuous feeding of molasses silage (grass, clover and alfalfa) was maintained during the duration of this experiment.

TABLE 2

A comparison between the actual production and normal expectancy of production per cow per day for animals on experiment II, on continuous feeding of molasses silage (grass, clover and alfalfa) as the only roughage

Week	Actual daily production*	Actual per cent decline	Expected normal daily production†
Initial	44.55	.....	44.55
1	44.29	0.6	43.66
2	45.97	−3.8	42.79
3	45.76	.5	41.93
4	43.81	4.3	41.09
5	43.19	1.4	40.17
6	42.73	1.1	39.37
7	41.68	2.4	38.58
8	39.97	4.1	37.81
9	40.82	−2.1	37.05
10	38.89	4.7	36.31
11	38.26	1.6	35.58
12	38.26	0.0	34.87

* Four per cent fat-corrected milk calculated according to Gaines (4).
† Calculated according to Landis (7) (2 per cent decline per week).

As this experiment was set up for ad libitum consumption of grass silage, it is significant to note the variation in consumption of grass silage by the different animals. This is shown as daily consumption of grass silage per animal per 100 pounds of live weight. These data, with the grain to milk ratio, are given in table 3 for each animal.

It is evident from the data in this table that the rate of consumption

among individual animals is very variable, and that, when the consumption per 100 pounds of body weight decreases, the grain to milk ratio must increase inversely in order to supply the necessary feed nutrients.

TABLE 3

Illustrating high grain feeding necessary to maintain production and body weight on a continuous feeding of molasses silage as the only roughage

Breed	Lb. grass silage consumption per 100 lb. live weight	Lb. of 4 per cent fat-corrected milk produced daily*	Lb. of 4 per cent fat-corrected milk produced for each lb. of grain fed*
Holstein	6.61	57.90	2.84
Ayrshire	6.02	36.49	2.38
Holstein	5.29	54.14	2.58
Holstein	6.13	38.35	2.26
Jersey	5.64	33.05	2.34
Holstein	3.87	48.99	2.13
Holstein	6.18	40.77	2.71
Ayrshire	4.68	29.82	2.21
Holstein	7.91	38.43	2.38
Guernsey	7.63	38.92	3.31
Shorthorn	5.96	44.75	3.25
Actual average	5.99	41.96	2.55

* Calculated according to Gaines (4).

EXPERIMENT III

The use of phosphoric acid was proposed for the preservation of green grass by Wilson (16). Because of the widespread publicity given to this method by commercial companies, and its adoption by many farmers, a single reversal feeding trial was planned to compare the value of a green crop preserved with molasses and one preserved with phosphoric acid when both were fed with corn silage and no hay.

The twelve milking animals selected for this experiment were divided into two balanced groups.

The rations used for this group of animals are designated as Rations I and II. They are as follows:

Ration I:

Corn silage—3 pounds for each 100 pounds live weight.

Molasses silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

Ration II:

Corn silage—3 pounds for each 100 pounds live weight.

Phosphoric acid silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

The corn silage was made from a crop that was well-eared and at a medium degree of maturity.

The molasses grass silage was preserved with 48.06 pounds of blackstrap feeding molasses per ton of green crop. The approximate per cent of each crop in the silage was as follows: grasses, 35; clovers, 40; alfalfa, 20; weeds, 5. The crop was cut early and stored in the silo with a minimum of drying. It was, however, felt that water would increase the value of the silage. Therefore, a three-fourth-inch stream of water was entered at the throat of the chopper. After the usual spoilage at the top, the rest of the silage came out in excellent condition.

The phosphoric acid grass silage (grass, clover and alfalfa) was preserved with 27.5 pounds of 68 per cent (food grade) phosphoric acid per ton of green material. The approximate per cent of each crop in the silage was as follows: grasses, 50; clovers, 25; alfalfa, 20; weeds, 5. The crop was cut early and stored in the silo with a minimum of drying. Water was added to this grass silage as it was to the molasses grass silage with a three-fourth-inch stream at the throat of the chopper. There was considerable spoilage at the top and around the edges through the entire depth of the silage. This was believed to be due to air leakage through a poorly constructed silo wall. Only good silage was selected and used to feed the experimental animals.

The grain mixture fed to both groups was a special 18 per cent protein, commercial, open formula mixed feed, as follows:

400 lbs.	wheat bran
400 "	ground corn or hominy
370 "	ground oats
300 "	fresh coconut oil meal
300 "	corn distillers' dried grains (not less than 10 per cent fat)
200 "	linseed oil meal, O.P.
20 "	steamed bone meal
10 "	common salt

In determining results the feed consumed and milk produced were totalled from the two groups when on each ration and reduced to the basis of one cow for one day. These final results are given in table 1.

These indicate that, with the conditions as represented in this experiment, there was little difference between the two rations. That is, grass silages preserved with molasses and grass silages preserved with phosphoric acid were equal in value as a supplement to corn silage.

EXPERIMENT IV

This experiment was designed much the same as Experiment III. Grass silage preserved with phosphoric acid was fed to one group, and grass silage preserved with molasses was fed to another group. The one important dif-

ference is that dry hay was used to supplement the grass silage, instead of corn silage. The object of the experiment was to compare the value of the two silages when fed with dry hay.

The ten milking animals selected for this experiment were divided into two balanced groups, A and B.

The rations were as follows:

Ration I:

Mixed hay—1 pound for each 100 pounds live weight.

Phosphoric acid silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture, according to production.

Ration II:

Mixed hay—1 pound for each 100 pounds live weight.

Molasses silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

The mixed hay was early cut and well cured. It was unofficially graded as follows: U. S. Grade No. 2 mixed hay. It contained timothy, red top, quack, alfalfa, blue grass, and red clover. Both the molasses and phosphoric acid grass silage were from the same silos as that used in Experiment III. The grain used was of the same mix as that used in Experiment III.

In determining results the feed consumed and milk produced were totalled from the two groups when on each ration and reduced to the basis of one cow for one day. These data are given in table 1.

These indicate that, with the conditions as represented in the experiment, there was little difference between the two rations. That is, grass silage preserved with molasses and grass silage preserved with phosphoric acid were equal in value as a supplement to mixed hay.

DISCUSSION OF RESULTS

Throughout these feeding experiments it was evident that the palatability of the silage depended on the quality of the crop ensiled, moisture content, and the preservative added.

These experiments indicate clearly that the production of the animals was not significantly different on the various combinations of roughage, providing the total nutrient intake remained constant.

When molasses grass silage was fed as the only roughage ad libitum in Experiment II, the consumption of nutrients in the form of roughage varied considerably with individuals. The average was 5.99 pounds of grass silage per hundred pounds, which furnished nearly the normally expected intake of nutrients in the form of roughage. The range, however, was from 3.87 to 7.91 pounds per hundred pounds live weight, which means that many animals will not consume so large an amount of dry matter in the form of

molasses grass silage as in combinations of more than one roughage as a succulent and dry feed. This necessitates the feeding of a greater amount of concentrates. In this regard it is significant to note that there was a great range in the rate of grain feeding. Certain animals consuming a small amount of grass silage were fed on a narrow grain ratio (as narrow as one pound of grain to 2.13 pounds of 4 per cent fat-corrected milk), while the others consuming a large amount of grass silage were fed on a wider grain ratio (as wide as one pound of grain to 3.25 pounds of 4 per cent fat-corrected milk). The average was one pound of grain to 2.55 pounds of 4 per cent fat-corrected milk. This was the narrowest grain to milk ratio of any group of animals included in the feeding experiments.

Although the production and body weights were satisfactorily maintained on a continuous feeding of molasses grass silage as the only roughage, it would not seem a practical feeding practice because of the decreased nutrient consumption in the form of roughage.

From Experiments III and IV there is evidence that the kind of preservative did not affect the feeding value of the silage, providing the total intake was maintained. There is also evidence to indicate that either phosphoric acid or molasses grass silage is a satisfactory supplement to corn silage or dry hay when fed under conditions as represented in these experiments.

When grass silage is used to replace all the dry hay, the ration may be deficient in vitamin D, and precautions should be taken against this deficiency when this feeding practice is continued over long periods. Similarly, when grass silage is used to replace corn silage, the nutrient intake may be lowered because of the lower percentage of total digestible nutrients in grass silage as compared with corn silage. This must be compensated for by increased consumption of either roughage or grain.

Grass silage usually contains a larger amount of protein than corn silage and, when used to replace corn silage, the protein content of the grain mixture may be lowered accordingly.

It must be remembered that grass silage is a variable product because of its moisture content, the crop from which it is made, and the manner in which it has been preserved.

SUMMARY AND CONCLUSIONS

Various combinations of molasses silage (grass, clover and alfalfa), corn silage and dry hay were fed to three groups of cows over a fifteen-week period. There was no significant difference in the production of the animals in the various groups, providing the total nutrient intake remained constant.

Molasses silage (grass, clover and alfalfa) was fed as the only roughage to eleven milking cows for twelve weeks. They maintained normal milk production and body weight. However, many of the animals were unable to consume the normal roughage nutrient intake on this diet. The decrease

in nutrient consumption was compensated for by an increase in the amount of grain.

In a ten-week single reversal feeding trial, phosphoric acid silage (grass, clover and alfalfa) was found to be equal in feeding value for milk production and maintenance of body weight to molasses silage (grass, clover and alfalfa) when the grass silages were used to replace corn silage in a normal ration of corn silage, mixed hay and grain.

TABLE 4

Chemical analysis of feeds used in experimental trials

Feed	Moisture	Protein	Ether extract	Crude fiber	Ash	Nitrogen free extract	Used in experiments
Corn silage	84.78	1.33	0.49	3.48	0.78	9.14	I
Corn silage	81.03	1.80	0.51	4.58	1.24	10.84	I
Corn silage	71.59	2.39	0.87	7.21	1.78	16.16	I
Molasses silage	80.25	1.36	0.53	6.27	1.01	10.58	I, II
Molasses grass silage	83.92	1.85	0.67	4.87	1.06	7.63	I, II
Molasses grass silage	74.52	2.76	1.14	7.68	1.58	12.32	I, II
Molasses grass silage	79.56	2.42	0.82	6.15	1.81	9.24	I, II
Mixed hay	5.41	8.74	2.02	28.87	5.96	49.00	I
Mixed hay	8.00	9.74	1.85	32.02	6.31	42.08	I
Mixed hay	6.21	10.91	2.30	28.52	6.81	45.25	I
Grain*	8.22	19.93	4.48	7.14	7.80	52.43	I, II
Corn silage	73.51	2.45	0.95	8.30	1.48	13.31	III
Molasses grass silage	65.41	6.33	1.18	11.08	2.90	13.10	III, IV
Phosphoric acid grass silage	70.21	2.71	0.80	11.24	2.00	13.04	III, IV
Grain*	9.77	19.37	5.40	8.94	6.24	50.28	III, IV
Mixed hay	8.27	15.34	1.76	35.02	7.39	32.22	IV

* Analyses according to Morrison (9) used in calculation.

In a twelve-week single reversal feeding trial, phosphoric acid silage (grass, clover and alfalfa) was found equal in feeding value for milk production and maintenance of body weight to molasses silage (grass, clover and alfalfa) when the grass silages were used to replace mixed hay in a normal ration of corn silage, mixed hay and grain.

TABLE 5

Digestion coefficients used (in per cent)

Feed	Protein	Ether extract	Crude fiber	Nitrogen-free extract	Experiment used	Reference
Corn silage	54.0	74.0	66.0	69.0	I, III	Morrison (9)
Grass silage	62.0	61.1	59.4	65.4	I, II, III, IV	Newlander *et al.* (10)
Mixed hay	59.0	58.0	51.0	69.0	I, IV	Morrison* (9)

* Used digestion coefficients for red clover hay all analyses.

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EFFECT OF PROTEOLYSIS ON LIPASE INDUCED RANCIDITY IN CHEDDAR CHEESE[1]

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Cheddar cheese made from milk to which a small amount of commercial lipase has been added will develop a flavor defect known as rancidity (1, 2). This effect is presumably due to lipolysis of butterfat and the consequent liberation of fatty acids, the lower members of which possess an unpleasant odor (3). Rancidity produced in this manner closely resembles the odor of rancid cheese which occurs occasionally but persistently under factory conditions. It has been suggested that milk lipase may be among the factors concerned in the development of rancidity under commercial conditions (4). Since lipases from various sources would be expected to possess many properties in common, the study of lipase induced rancidity is of interest in connection with the rancid cheese problem.

It has been known for some time that lipase is protein in nature (5), and that as such it is subject to inactivation by proteolysis (6). These observations have been extended by their application to the study of cheese flavor defects. In this investigation experimental evidence is submitted to show that rennet or pepsin or both inactivate, to some extent, added lipase in cheddar cheese and that the use of higher amounts of these proteolytic enzymes contributes to flavor improvement of such cheese.

EXPERIMENTAL

Milk was obtained from the Experimental Farm herd. Small vats of cheese were made using 240 pounds of milk. This gave two 10–12 lb. cheese to each vat. Cheese were made by an experienced maker according to standard procedures except as otherwise indicated below. They were stored at 60° F. until the first grading and then transferred to a second storage held at 48–50° F. The cheese were graded at intervals for flavor only by an experienced member of the Dominion Grading Staff.

It was first necessary to determine the amount of commercial lipase (Pfanstiehl) which when added to cheese milk would develop rancidity in 10 to 14 days. This was done by making a series of vats of cheese using varying amounts of lipase. It was found that 2.5–5.0 gms. of lipase per 1000 pounds of milk gave a satisfactory working level.

It was possible to show in preliminary experiments that higher than the

Received for publication February 4, 1941.

[1] Contribution from the [3] Division of Bacteriology and Dairy Research, Science Service. (No. 122 Journal Series); [2] the Division of Chemistry, Science Service, and the [4] Division of Animal Husbandry, Experimental Farms Service.

usual amount of Hansen's rennet (*e.g.* 6 oz. per 1000 lb. milk) retarded to a certain extent the development of rancid flavor by lipase in cheese. The same effect was demonstrated for pepsin and for mixtures of rennet and pepsin. Pepsin from two sources was used; Armour's pepsin made for cheese making during the last war, and Merck's soluble pepsin. These are referred to later as pepsin A and pepsin M, respectively. Papain and trypsin (both Eimer and Amend products) were also tried but, owing to a bitter licorice-like flavor of the former and a high lipase content of the latter their use was discontinued.

The process of making cheddar cheese is not a rigid procedure. Such factors as the daily variation in the composition of milk, the starter culture, the rate of acid development, and the details of the manufacturing process are difficult to control exactly under experimental conditions. These make the interpretation of the results of grading rather difficult. In order to eliminate these variables the following method was adopted. In the morning all milk was pooled, brought to temperature and the starter culture added. When nearly sufficient lactic acid had developed, the milk was divided into two equal portions. To each vat was added an equal amount of lipase suspended in water. One vat was coagulated with a low amount of rennet while the other received a higher amount than normal and the make was then completed in the usual manner. Pepsin alone, and pepsin and rennet mixtures were also used instead of rennet. In this way each vat was as nearly identical as possible in every respect except in the amount of proteolytic enzymes used. Accordingly, one vat served as a comparison or reference standard for the other. Variation in the flavor score could then be attributed mainly to the effect of proteolytic enzymes on added lipase in the cheese.

The results are summarized in the accompanying table. Comparison vats (or mutual reference standards) as explained above, are indicated by A and B following the same number. The cheese of series A have in each case a lower content of proteolytic or coagulating enzymes than the corresponding members of the parallel B series. The amounts of rennet, pepsin and lipase are shown in the second column. The succeeding columns show the successive flavor scores on the respective cheese at indicated intervals.

An examination of the results shows that in general cheese of the B series have a higher flavor score than their corresponding mates of Series A. Since the cheese of the B series have also a higher content of proteolytic enzymes it is concluded, therefore, that proteolytic enzymes enhance cheese flavor by preventing the onset of rancidity due to added lipase.

DISCUSSION

In order to assess the value of the above experimental data accurately, careful consideration must be given to the various factors involved.

It will be noted that there are a few exceptions to the general statement

TABLE 1

Rennet, pepsin, lipase and flavor of experimental cheese

Cheese	Treatment per 1000 lbs. of milk			Flavor scores[1] of cheese during curing									
	Lipase	Rennet	Pepsin	Age in days	Score	Age in days	Score	Age in days	Score	Age in days	Score	Age in days	Score
	gm.	*oz.*	*gm.*										
1 A	5	2		9	38	16	38-	23	37-	86	37		
1 B	5	3¼		9	39-	16	38+	23	36	86	37		
2 A	2.5	2		11	38	18	38	32	36-	60	39-		
2 B	2.5	6		11	38+	18	39-	32	38-	60	39		
3 A	2.5	2		10	38-	17	37	31	37-	59	36		
3 B	2.5		86M	10	39-	17	38-	31	38-	59	36+		
4 A	5	3¼		11	38-	18	37	25	36	88	36		
4 B	5	3	30A	11	39	18	37-	25	36+	88	37-		
5 A	5	3¼		10	38	17	38	24	36	45	36	87	38-
5 B	5	3	43M	10	39-	17	38	24	38-	45	38	87	38+
6 A	2.5	3		9	38	16	38+	30	36	58	37+		
6 B	2.5	3	43M	9	39-	16	38	30	38	58	37		

[1] Flavor standards for grades of Canadian cheddar cheese.

First grade cheese—minimum score for flavor 39
Second " " " " " " 37
Third " " " " " " < 37
unless below third grade.

that the flavor scores on cheese in series B are higher than those of corresponding cheese in Series A. No explanation is offered for this discrepancy. In any event these exceptions do not invalidate the general conclusion.

Also, admittedly, some of the differences between the flavors on corresponding cheese are small. However, because of the system of comparison vats more significance can be attached to these results than could otherwise have been possible. The variations between any two different gradings is subject to some uncertainty because of the variation in the judgment of an individual grader at two periods remote from each other. However, differences in this direction are of secondary importance in this investigation.

It must also be borne in mind that if, on the one hand, larger amounts of lipase than the above established level are used, rancidity in cheese is developed to a greater intensity than that encountered commercially. The lipolysis of cheese fat is extensive during the initial stage and the beneficial effect of proteolytic enzymes cannot undo the damage already in existence. On the other hand, when smaller amounts of lipase are used in order to decrease the rate of lipolysis so that the remedial effect of rennet or pepsin or both can be established early, the differences between the flavor scores on

any two corresponding cheese are reduced. The interpretation of results is thus made more difficult. In obtaining the above data it was therefore necessary to select an arbitrary working level of moderate sensitivity.

The differences in the flavor score between corresponding cheese of the series A and B are quite consistent at the period of their first grading. This observation is of some importance because cheese are generally graded at 10–14 days in commercial practice. Although the results of subsequent gradings have also been included and the same general trend is shown, the maintenance of a high score flavor over prolonged periods of storage can be considered as a problem separate from that of the initial flavor scores.

Mention might be made of the types of flavor obtained in the above experimental cheese. It is possible to obtain typical butyric rancidity particularly in those cheese containing higher than the adopted lipase level. With lower amounts of lipase the flavors were often described as not straight rancid, unclean, dirty, etc. It has been pointed out in our preliminary communication (4) that these flavors resemble those known to occur commercially. Therefore, it has been suggested that lipase may not only be responsible for the rancid flavor but to some extent for certain less defined flavor defects.

Assuming that naturally present milk lipase does play a part in the development of cheese flavor (7) the above results suggest an additional role for the proteolytic enzymes in cheese. In addition to its coagulating function with its contingent effect on texture, and the proteolysis of casein or chemical ripening, rennet may play an active part in the inactivation of lipase, which when present in abnormal amounts in cheese milk would be expected to produce undesirable flavors.

Lipase induced rancidity has not been definitely identified with the rancid flavor occurring under factory conditions. Other factors may also be involved. The results of this investigation, however, are submitted because of a possible existing relationship. At the same time additional information on the proteolytic function of rennet has been obtained. Further study of lipase and its possible relation to rancidity in commercial cheddar cheese is being continued.

SUMMARY

Rancid and other less defined flavors have been reproduced in cheddar cheese by the addition of commercial lipase to cheese milk.

A higher flavor score was obtained in cheese to which lipase was added when higher amounts of pepsin or rennet or both were used in its manufacture than in corresponding cheese where smaller amounts of proteolytic enzymes were added.

An additional function has been attributed to the rennet enzymes.

ACKNOWLEDGMENT

The authors are indebted to Mr. T. J. Hicks, Senior Dairy Produce Grader, Dairy Products Division, Department of Agriculture, for his services in grading the experimental cheese.

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UTILIZATION AND EXCRETION OF ASCORBIC ACID BY THE DAIRY COW*

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The results of early studies (1, 2, 3) concerning the influence of diet upon the antiscorbutic potency of cow's milk, appeared to indicate that the vitamin C content of the milk paralleled that of the ration. During the same period, however, findings were reported (4) which led to the opposite conclusion, namely, that the ration received by cows had no influence on the antiscorbutic potency of their milk.

Since the development of chemical methods for the quantitative determination of the antiscorbutic factor, which was shown to be ascorbic acid, further differences of opinion have arisen concerning the factors which influence the vitamin C content of cow's milk. As a result of recent work (5, 6, 7) the influence of breed and stage of lactation have been emphasized. Several workers (5, 8, 9) have attributed variations in the ascorbic acid content of milk to the season of the year. Other investigators (10, 11, 12, 13, 14) have concluded that the vitamin C content of cow's milk tends to be quite constant and is independent of the ration of the cow. Opposed to this view are those who still contend that the ascorbic acid content of the diet is a factor which cannot be ignored (6, 15).

A number of investigators have suggested that the cow is able to synthesize vitamin C (9, 13 ,16, 17, 18, 19). How or where this synthesis occurs is not understood, although one investigator (30) has claimed that the vitamin C of cow's milk is synthesized by the udder parenchyma and that the synthesis depends largely upon the condition of the udder.

The failure of numerous experiments involving standard dairy rations to establish clearly and conclusively the importance of the ascorbic acid content of the diet with regard to the amount of the vitamin in the milk, made it seem desirable to repeat previous work, but to alter the procedure by supplementing standard rations with known amounts of pure synthetic ascorbic acid. Moreover, it was desired to increase the significance of customary milk ascorbic acid analyses by determining, simultaneously, the amount of ascorbic acid in the blood and in 24-hour samples of urine.

Received for publication February 11, 1941.
* Authorized for publication on January 21, 1941, as paper No. 1016 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

If, in the above proposed studies, it could be shown conclusively that the vitamin C content of milk is independent of the ration of the cow, it was hoped that some explanation could be obtained for the anomalous results just mentioned. It was desired, for example, to find new evidence for the synthesis of ascorbic acid in the cow. Further, it was hoped that some clue might be found regarding both the metabolic fate of ascorbic acid and the factors which influence its elimination from the body in the milk and in the urine.

EXPERIMENTAL

Methods and Apparatus. One or both of two chemical methods of estimation were used in all ascorbic acid analyses. These were the Tillmans titration method (20) employing standard 2,6-dichlorobenzenoneindophenol, and the newer Roe furfural method (21), which estimates total ascorbic acid (both reduced and reversibly oxidized ascorbic acid).

Milk samples were taken in a special apparatus which has been shown to preserve all of the ascorbic acid of the milk in the reduced form (22). The use of this apparatus permitted the quantitative determination of the vitamin by the convenient indophenol titration method.

Blood samples were taken in the conventional oxalated tubes under paraffin oil. For each test, 20 to 30 ml. of blood were taken from the jugular vein of the cow. As soon as the sample was obtained, the collection apparatus was placed inside a dark glass receptacle containing ice and water and was taken immediately to the laboratory for analysis. Essentially the Farmer and Abt macro-method (23) for plasma ascorbic acid was used for routine blood analysis.

Successful collections of the total 24-hour urinary excretion, free from fecal contamination, were made by employing a special rubber urine tube developed by Forbes and coworkers (24). The 18-liter carboys, which were used as receivers in the collection apparatus, were painted black to exclude light and were charged with enough glacial acetic acid to give a final concentration of about 5 per cent by volume. Addition of stick metaphosphoric acid to the acetic acid appeared to give no better results than when acetic acid was used alone; consequently, the addition of metaphosphoric acid was discontinued. In urinary ascorbic acid analyses, both the indophenol titration and the Roe furfural method were employed, with the exception of the earliest work on one of the cows which was done before the furfural technique had been reported. This double analysis seemed desirable in view of the lack of unanimity among various workers concerning the specificity of present methods for the determination of ascorbic acid in urine. Moreover, it appeared probable that some of the vitamin would be unavoidably oxidized to dehydroascorbic acid during the collection of a 24-hour sample. Such has since been shown to be true with human urine (25). The furfural method

is particularly valuable in this case because it determines both dehydro- and reduced ascorbic acids.

It was recognized at the outset that it would be desirable to eliminate from the projected work the effect of breed differences by limiting the experiments to one breed of dairy cow. Holstein cows were eventually chosen, largely because they were available for experimental purposes at the time this work was started.

Effect of a Standard Ration. In order to have values which might later be used to compare with those obtained during administration of ascorbic acid, each cow was maintained on a standard ration for an interval of time during which analyses were made to determine the concentrations of ascorbic acid in the blood, the milk, and the urine. The averages of the values obtained during typical five-day test periods are given in table 1. Five-day

TABLE 1

*Summary of results obtained when Holstein cows were fed a standard ration and when they were fed a standard ration supplemented with ascorbic acid**

	S. R.	C. S.	G. C.	D.
Mg. ascorbic acid per ml. milk	0.019	0.021	0.021	0.020
Mg. ascorbic acid per 100 ml. plasma	0.53	0.58	0.50	0.48
Mg. ascorbic acid per day in urine—				
Indophenol titration	45.7	52.7	54.1	26.8
Furfural method	910.5	833.1	643.5	

S. R.—Standard ration.

C. S.—Standard ration supplemented with 50 to 100 grams of ascorbic acid mixed with corn silage.

G. C.—Standard ration supplemented with 50 grams of ascorbic acid in gelatin capsules.

D.—Standard ration supplemented with 50 grams of ascorbic acid administered by drenching.

* The values given in Table 1 represent the averages obtained in experiments employing from two to four cows, except in the case of the ascorbic acid administered by drenching, where the values are from an experiment with a single cow.

sampling periods were considered representative for any diet, in view of the fact that all cows received a particular ration for several days, or even weeks, prior to the actual collection of samples.

Effect of Ascorbic Acid Added to Corn Silage. In choosing methods for administration of massive amounts of ascorbic acid, it was decided to administer some of the vitamin mixed with a small amount (3–5 pounds) of corn silage. It was found that 50–200 grams (1,000,000–4,000,000 International Units) of ascorbic acid placed in such a mixture were consumed by a cow in a period of about 10 minutes. In contrast, ascorbic acid mixed with grain was eaten very slowly, if at all.

Experimental periods were designed to include a two-day interval during which the cow received the standard ration alone, followed by a three-day period during which she received the standard ration supplemented each day with a certain amount of crystalline ascorbic acid mixed with corn silage,

which was succeeded by a post-administrative period of two days during which the animal again received only the standard ration. The same procedure was used in subsequent experiments during which the ascorbic acid was administered in gelatin capsules and by drenching. The data from such experimental periods are summarized in table 1.

From a comparison of values in table 1, it is apparent that supplements of ascorbic acid administered by three different methods had no significant influence upon the concentrations of ascorbic acid in the blood, milk, or urine of the cows used in these experiments.

Effect of Ascorbic Acid Injected Intravenously. Rasmussen and coworkers (26) reported that intravenous injection of ascorbic acid resulted in a marked temporary rise in the vitamin C content of the milk of the ewe and cow. It was desired to repeat this work and to enlarge upon it by making analyses of blood and urine as well as of milk. Therefore, experiments were performed during which ascorbic acid was injected into the blood stream via the jugular vein. 24 grams of crystalline ascorbic acid, dissolved in sterile water, were given in this manner on each of two or three successive days. The effect of these ascorbic acid injections was studied with three cows with corresponding results. Typical values are given for one of the cows in table 2.

TABLE 2

Concentration of ascorbic acid in the blood, the milk, and in the urine of a Holstein cow during feeding of a standard ration supplemented with intravenous injections of ascorbic acid

Day	Mg. ascorbic acid per 100 ml. plasma	Mg. ascorbic acid per ml. milk	Mg. urinary ascorbic acid per day	
			Indophenol titration	Furfural method
1	0.58	0.021	24.3	611.1
2	0.58	0.020	34.6	241.5
3*	2.74**	0.020 0.019 0.022	6922.9	13311.8
4*	4.42**	0.024 0.028 0.028	9870.0	13632.6
5*	4.86**	0.030 0.029 0.027	14715.2	18194.0
6	0.86	0.028 0.025 0.022	8813.6	20923.8
7		0.021	161.2	604.7
8		0.020	117.8	982.8

* 24 grams of ascorbic acid dissolved in 100 ml. of sterile water were injected into the jugular vein. The multiple values for milk ascorbic acid represent each of the three daily milking periods.

** Blood analyses were made on samples taken 1½ hours after injection of ascorbic acid.

The intravenous injection of ascorbic acid produced unmistakable increases in the concentration of that vitamin in the milk and urine as well as in the blood. A very large portion of the injected ascorbic acid which could be accounted for appeared in the urine. In the case of one cow, this amounted to over ninety per cent of the total ascorbic acid injected. These results correspond closely to those recently reported (31) for experiments in which ascorbic acid was injected into goats.

Effect of Ascorbic Acid Injected Subcutaneously. The experiments described thus far seemed to indicate that ascorbic acid administered by non-injection methods never reached the blood stream in significant amounts. Further, data from the intravenous injection experiments showed that the concentration of ascorbic acid in the milk was definitely increased if large amounts of the vitamin reached the blood stream. In order to further substantiate these findings, it was desired to administer some ascorbic acid by a method which would not place the vitamin directly in the blood stream but which would insure the ultimate arrival of large amounts in the circulation. Such a purpose was accomplished by setting up an experiment in which ascorbic acid was injected subcutaneously in regions around the cow's forelegs.

The effect of the subcutaneous injections of ascorbic acid closely resembled that of the intravenous injections. The influence of the subcutaneous injections upon blood, milk, and urinary ascorbic acid values was more gradual and somewhat less pronounced than in the case of the intravenous injections. During the course of three successive daily injections of 24 grams of ascorbic acid, the ascorbic acid titer of the milk was raised from 0.020 to 0.027 mg. per ml. and that of the urine from 600 to a peak of 15,000 mg. per day.

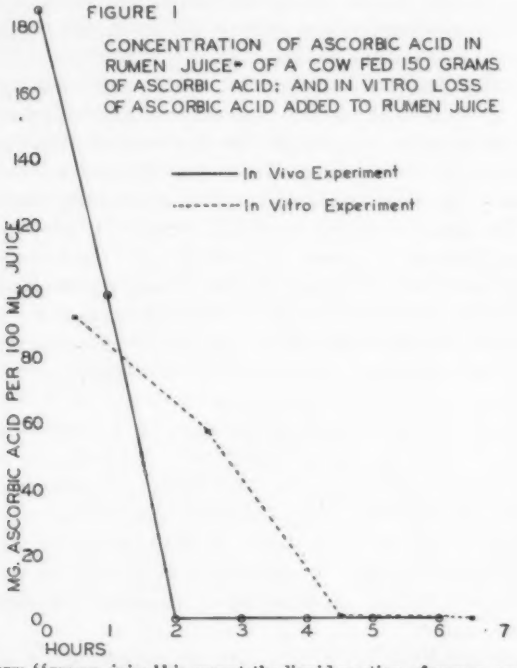
Studies with a Rumen Fistula. At this point in the experiments it appeared to be almost certain that the failure of massive amounts of ingested ascorbic acid to influence the concentration of the vitamin in the milk or to significantly alter its concentration in the blood and urine, could be attributed to a destruction of this substance in the rumen. To investigate this possibility, a rumen fistula was created in one of the cows. With this permanent opening leading directly into the largest compartment of the cow's stomach, it was possible to study the fate of ingested ascorbic acid by removal and analysis of partially digested food at intervals after feeding.

A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after feeding supplements of ascorbic acid and after insertion of the vitamin directly into the rumen. During such periods, the average concentrations of ascorbic acid in the blood plasma, in the milk, and in the urine were respectively 0.42 mg. per 100 ml., 0.019 mg. per ml., and 1260.5 mg. per day, values which do not differ significantly from those obtained during feeding of a standard ration alone.

The disappearance of ascorbic acid from the rumen contents during an experiment in which 150 grams of ascorbic acid were fed is shown graphically in figure 1. A preliminary report on this experiment has been given elsewhere (32).

FIGURE 1

CONCENTRATION OF ASCORBIC ACID IN RUMEN JUICE* OF A COW FED 150 GRAMS OF ASCORBIC ACID; AND IN VITRO LOSS OF ASCORBIC ACID ADDED TO RUMEN JUICE



* By the term "rumen juice" is meant the liquid portion of rumen contents.

In order to demonstrate that volume changes were not responsible for the pronounced decrease in the ascorbic acid concentration of the rumen contents as shown above, an experiment was performed in the laboratory with a controlled volume of rumen contents. 1000 ml. of rumen contents, from which the coarse particles of feed had been removed by straining through cheesecloth, were placed in a dark-glass, wide-mouth bottle. To this mixture, which had a pH of 6.50, was added 1000 mg. of crystalline ascorbic acid. After thoroughly mixing the contents, the bottle was loosely stoppered and placed in a water bath maintained at a temperature of 39° to 42° C. At intervals, samples were removed for analysis.

Figure 1 shows that the ascorbic acid disappeared just as it had in the *in vivo* experiments with the exception that the *in vitro* decrease proceeded at a more gradual rate. This slower rate of destruction of ascorbic acid in the *in vitro* experiment as compared to the *in vivo* experiments seems readily

explained by the absence of the continual stirring and the circulation of gases so characteristic of the rumen.

DISCUSSION OF RESULTS

State of Ascorbic Acid in Urine. As soon as the furfural method was applied to the urine analyses it became apparent that the results obtained ranged from one and a half to thirty times as high as those obtained by the indophenol titration. During injections of ascorbic acid, however, this discrepancy narrowed to a point where, in some cases, the results obtained by the two methods almost coincided. These facts, together with the knowledge that the indophenol and furfural methods of analysis checked well when applied to 24-hour samples of rat urine collected under similar conditions (33), suggested that important amounts of the ascorbic acid excreted daily by a cow on a standard ration were excreted in the form of dehydroascorbic acid. In order to test this hypothesis, samples of urine were collected as they were excreted and analyzed at once. Table 3 gives the results obtained

TABLE 3

Reduced and oxidized ascorbic acid in cow's urine immediately following urination

Cow No.	Reduced ascorbic acid Mg. per liter Indophenol titration	Total ascorbic acid Mg. per liter Furfural method
1	20.9	17.0
2	33.2	25.4
3	19.7	18.2
4	10.1	10.3
5	27.7	25.1
6	27.6	25.4
7	53.1	48.1
8	40.1	43.3
9	21.5	19.0
10	31.0	29.6

from Holstein, Jersey, Brown Swiss, and Ayrshire cows. These findings seem to indicate that all the ascorbic acid excreted in the urine of the dairy cow is in the reduced form. The greater values obtained in eight out of ten cases by the indophenol method are readily explained by the recognized tendency to go beyond the endpoint, which is obscured to an extent proportional to the concentration of the urine pigments. The greater values obtained by the furfural method for 24-hour samples of urine must indicate, therefore, that variable amounts of the excreted ascorbic acid are oxidized to dehydroascorbic acid during the interval between excretion and analysis, or that the urine contains appreciable amounts of non-ascorbic acid furfural precursors capable of forming a derivative with 2,4-dinitrophenylhydrazine. Tests for the latter interfering substances were made repeatedly and in no case showed the presence of concentrations sufficiently large to interfere with

the method. Consequently, it may be concluded that while all the ascorbic acid excreted in the urine is originally in the reduced form, some of it is oxidized to dehydroascorbic acid under the conditions involved in the collection of a 24-hour sample of urine.

Destruction of Ascorbic Acid in the Rumen. Several explanations might be given for the rapid and pronounced disappearance of ascorbic acid from the rumen after oral administration of massive amounts of the vitamin. It might be argued that the large amounts observed in the first two or three samples represented stages in the incomplete mixing of the ascorbic acid with rumen contents. Changes in the concentration of the rumen ascorbic acid could also be attributed to an intake of water by the animal, or by a passage of a portion of the rumen contents to other chambers of the stomach.

Another interpretation of the findings is that the very soluble vitamin was rapidly absorbed. This has been suggested by Riddell and Whitnah (27). These workers studied the fate of vitamin C in the rumen contents of a cow with a rumen fistula and in a steer at slaughter. In each case the rumen contents were found to contain less than one-tenth the vitamin C of green rye ingested twelve hours earlier. These workers explained the rapid disappearance of ascorbic acid from the rumen by suggesting that ascorbic acid was rapidly absorbed. The basis for this suggestion was the observation of a temporary doubling of the vitamin C content of the blood within 12 hours and a fivefold increase in the ascorbic acid content of the urine within 60 hours after green feed was first supplied.

The most plausible explanation, however, and the one demanded by our experimental data, is that a rapid and pronounced destruction of ingested ascorbic acid occurs in the rumen. Thus, while the other factors which have been mentioned undoubtedly have some influence on the results observed, it is hardly likely that they account for changes of the magnitude indicated in figure 1.

The incomplete mixing theory is especially inadequate. When a cow is eating, the rumen contracts about three times per minute and each contraction causes a flow of liquid throughout the rumen and its solid contents. While figure 1 indicates that the first sample was removed at zero time, it was actually taken about five minutes after the animal had eaten the last of the ascorbic acid treated silage or 15 minutes after she had started to eat. This means that the ascorbic acid had been subjected to 15 to 45 contractions of the rumen. Further, when the cow was slaughtered, it was found that the rumen contained 38 liters of liquid or semi-liquid material. If 150 grams of ascorbic acid were thoroughly distributed throughout this liquid, there should be a concentration equivalent to about 400 mg. per 100 ml. of juice. Figure 1 indicates that the concentration of ascorbic acid in the juice, as shown by analysis of the first sample removed, was about 185 mg. per 100 ml. of juice. From these facts, it would appear that fairly complete mixing had occurred, accompanied by extensive destruction of the vitamin.

Observations of this and other dairy cows show that they drink water infrequently. It is difficult to say how much and how often liquid material passes permanently from the rumen into other compartments of the stomach. Ewing and Wright (28), working with steers, found that the average rate of passage of food residues through the rumen and reticulum was 61 hours. In any event, credence in the volume-change explanation for the disappearance of ascorbic acid from the rumen is seriously discounted by the results of *in vitro* constant-volume experiments such as shown in figure 1.

Indirect evidence that ingested ascorbic acid is largely destroyed in the rumen rather than being rapidly absorbed is given in the blood, milk, and urine ascorbic acid values, which, with the possible exception of the urine, show no response to the feeding of 50 grams or more of ascorbic acid. Why there is always a small amount of ascorbic acid in the rumen, as there appears to be, is difficult to explain. Possibly the release of this vitamin from solid feed fragments proceeds gradually and at a rate slightly higher than the rate of destruction. We hope to continue the study of the factors responsible for the destructive effects described.

In the light of the demonstrated destruction of ascorbic acid in the rumen, it becomes clear why various methods of oral administration of the vitamin failed to produce a response in the milk or other body fluids. If the ascorbic acid administered in gelatin capsules had been able to survive rumen conditions and reach other compartments of the stomach, it seems likely that other results might have been obtained, for the ascorbic acid content of the milk of non-ruminating animals, *e.g.*, guinea pigs (26) and humans (8, 29), has been shown to be influenced by diet. In work with the rumen fistula, however, it was found that gelatin capsules were dissolved and the ascorbic acid was released after 10–15 minutes in the rumen.

SUMMARY

1. Special equipment was employed which permitted the complete collection from dairy cows of 24-hour samples of urine free from any fecal contamination. It was found impractical, if not impossible, to preserve all the ascorbic acid in such urine samples in the reduced form.

2. By the simultaneous application of the indophenol titration and the furfural method of analysis to freshly excreted samples of urine, it was possible to show that ascorbic acid is excreted in cow's urine in the reduced form.

3. Analysis of over 50 samples of blood obtained from four Holstein cows showed that the ascorbic acid content of the plasma ranged from 0.43 to 0.62 mg. per 100 ml. when the cows received standard dairy rations.

4. Ascorbic acid was administered to Holstein cows (a) mixed with a small amount of corn silage, (b) in gelatin capsules, (c) in aqueous solution, (d) intravenous injection, and (e) by subcutaneous injection. Administration of as high as 100 grams (2,000,000 International Units) of ascorbic acid per day for three days by a non-injection method failed to increase the ascor-

bic acid concentration of the milk or blood and had only a slight effect on the concentration of the vitamin in the urine. It was only by the injection methods that a significant increase in the ascorbic acid concentrations of the blood, milk, and urine could be demonstrated. The greatest increase in milk ascorbic acid concentration during experiments in which 24 grams of ascorbic acid were injected intravenously on each of three successive days, was from 20 mg. per liter to 30 mg. per liter.

5. A rumen fistula was made in a Holstein cow. Experiments were performed in which this cow was fed as much as 150 grams (3,000,000 International Units) of synthetic ascorbic acid at one time; similar amounts were also placed directly in the rumen through the fistula opening. A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals. Ascorbic acid added to rumen contents *in vitro* and stored in a dark-glass, stoppered receptacle at 39°–42° C. disappeared at much the same rate as that of the *in vivo* experiments. In making analyses, both the indophenol titration and the Roe furfural method were employed.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Dr. J. F. Shigley, who performed the fistula operation, and to express their appreciation for the generous supply of ascorbic acid furnished by Chas. Pfizer and Company, New York.

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STORAGE OF DAIRY BULL SPERMATOZOA*

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An appreciation of the limitations and possibilities of storing dairy bull semen and retaining its fertility is necessary in order to define the practical limits of artificial insemination with shipped or stored semen. While various diluents have been proposed and different storage temperatures have been suggested, there still remain many unexplained factors concerning the successful storage of dairy bull semen. One difficulty in semen storage research has been the lack of an accurate criterion of fertility. Since the time of survival with vigorous motility of bull spermatozoa stored undiluted at 40° F. has been shown to be correlated with the fertility of bull semen (14), this property of the semen may be used in evaluating storage methods more accurately than simple determination of time of survival.

Refrigeration has been recognized for some time as the most effective method of preserving bull semen (15). Spermatozoa normally live only a short while at body temperature, and room temperature (70° F.) has likewise been found too high for successful semen storage (17). The highest temperature at which spermatozoa may be stored successfully has been given as 50° F. (4), and longer viability was secured with reduction of the storage temperature to 35° to 40° F. (4, 7, 8, 17). It has been emphasized that the change of temperature of the semen must be gradual or irreversible immotility will result from temperature shock (2, 6).

Many diluents of various composition have been used with bull semen, but their main use has been in increasing the volume (15, 4). The addition of nutrient substances to these diluents did not increase the survival of spermatozoa in them (1). The buffering action provided by some diluents has increased the motility of spermatozoa (8). A diluent using egg yolk plus a buffer has been reported as causing a marked increase in survival and fertility of stored bull semen (11).

The secretions of the accessory sex glands were shown to be harmful to survival of spermatozoa and were entirely unsuitable as diluting fluid for bull semen (5, 10, 11). With horse and boar semen the removal of the sperm fluid and concentration of the spermatozoa has resulted in greater survival in storage (16, 9). Experiments along this line with bull semen were not successful in obtaining greater survival in storage.

Early investigators excluded air from the semen by a layer of paraffin

Received for publication February 18, 1941.

* Contributed by the Department of Dairy Husbandry, Missouri College of Agriculture, Journal Series No. 729.

oil (15). Recent work has shown that such procedure is not necessary and that spermatozoa live longer with free access to oxygen (12, 13).

Although there are records of cows being settled with semen eight days old, the practical limits of storage of bull semen have been given as only 12 to 30 hours (3).

This paper presents the results of studies to find the most practical method of storing bull semen so as to preserve its fertility. Investigations were made as to the desirable temperature for storage, the benefits to be derived from using various diluents, the possibility of removing the accessory sex gland secretions to improve survival, and the actual effectiveness of stored semen as measured by its fertility when used in artificial insemination.

EXPERIMENTAL AND RESULTS

The semen used in these experiments was collected by use of the artificial vagina during the winter and spring of 1940 from ten bulls in the University of Missouri dairy herd.

Motility determinations were made at 250× magnification of a drop of semen placed on a microscope slide in a stage incubator at 100° F. Motility was rated from 0 to 5. No motility was 0, and 5 represented the very best grade of motility. Weak, oscillatory motion with less than 40 to 50 per cent of the spermatozoa moving was rated 1. Progressive degrees of motility from 1 to 5 were rated 2, 3, and 4. Unless otherwise stated, all storage was made in an ordinary electric refrigerator operating at about 40° F. As soon after collection as possible, nearly always less than 30 minutes, the semen was put in small sterile glass vials, stoppered with freshly paraffined corks, wrapped in paper towelling, covered with rubber cots, and placed in a tray of water at 40° F. in the refrigerator. Cooling was thus made gradual and was accomplished within an hour after collection. A part of each sample was stored untreated at 40° F. as control for part of the same sample which was used in the experiment.

Effect of temperature on viability of semen. The storage temperature was observed to have a marked effect upon the rate of loss of motility. Thirty-seven samples representing ten different sires were stored at 70° to 75° F., while duplicate samples of the same semen were stored at 40° F. The averages of the motility ratings of these samples at various storage periods are presented in figure 1. At the end of six hours the semen stored at 70° F. showed slightly more vigorous motility than did that stored at 40° F. After 16 hours storage, motility in samples stored at 40° F. was much superior to that in samples stored at 70° to 75° F. Sixteen of the 37 samples stored at 70° to 75° F. were non-motile at 24 hours, and at 40 hours all except one were dead, while semen from the same samples stored at 40° F. still had good, vigorous motility.

A few samples of semen were stored at 31° to 32° F. Motility ratings of semen stored at this temperature fell more rapidly than motility in semen stored at 40° F., but the spermatozoa were still viable by the time motility rating in semen stored at 40° F. was reduced to 1. Thus, although motility was reduced, the time of survival was not greatly reduced.

Effect of diluting solutions on sperm viability. Samples of semen were divided to compare the effect upon viability in storage when diluted with

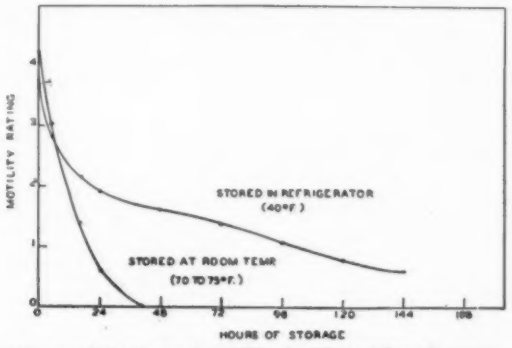

FIG. 1. Average maintenance of motility in 37 samples of semen stored at 40° F. and 70° to 75° F.

various diluents. The dilution rate used was three parts of diluent to 1 part of semen. The first diluents tried were 3 and 5 per cent glucose solutions and Milovanov's (10) S-G-C-2 dilutor. All of these were definitely harmful to the spermatozoa as measured by their effect upon motility maintenance. Motility was more vigorous in the undiluted semen in every case although the time of survival did not differ greatly between the diluted and undiluted semen.

The egg-yolk buffer (E-Y-B) dilutor which was proposed by Phillips *et al.* (11) was used in 18 samples of semen from eight different bulls. The comparison of the part of these samples diluted with E-Y-B dilutor with the part stored undiluted is shown in figure 2. On the average the use of this diluent was definitely favorable to sperm motility up to the fifth day of storage.

Thereafter, the semen which was diluted perished rapidly while that which was not diluted remained motile to a low degree. The effect of the E-Y-B dilutor varied greatly as to the semen of different bulls in which it was used. Semen from two bulls which was quite viscous and highly concentrated was greatly benefited by dilution with E-Y-B dilutor. Semen of these bulls was normally of very good initial motility, but the undiluted semen became very viscous in storage and motility declined rapidly. Semen of the other bulls which was more nearly normal in characteristics was

benefited very little if any by the use of this dilutor. In only one case, however, was the motility rating in the E-Y-B diluted semen less than that in the undiluted semen until the fifth day of storage. When the E-Y-B dilutor was added to undiluted semen which had become of low motility in storage, no reactivating or stimulating effect was secured even though a part of the same sample which had been stored diluted with E-Y-B dilutor initially had good motility at the time. In storage periods exceeding five days, the undiluted fractions of the semen survived longest.

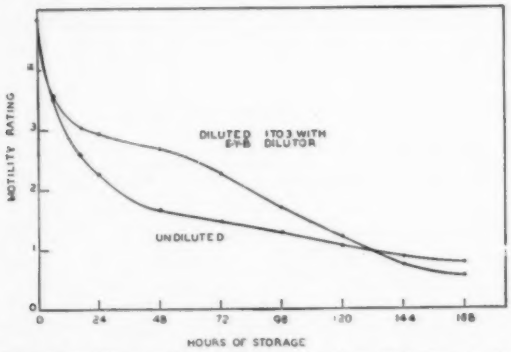

FIG. 2. Average motility ratings of semen stored undiluted and of semen from the same samples diluted 1 to 3 with egg-yolk buffer dilutor.

Effect of removal and replacement of the sperm fluid upon motility and survival of spermatozoa. Since the secretions of the accessory sex glands had been shown to be harmful to survival of the spermatozoa (5, 10, 11), the effect of removal of part of these secretions was studied. Separation was accomplished by centrifuging the fresh semen at 1300 RPM for 15 minutes. The relative centrifugal force was approximately 375 grams. The supernatant fluid which was clear and practically devoid of spermatozoa was removed by use of a pipette.

Spermatozoa stored in the concentrated state could not be reactivated with physiological saline or five per cent glucose solutions after 48 hours of storage, while the same semen untreated showed very vigorous motility at 48 hours. Replacing the sperm fluid with S-G-C-2 dilutor or three per cent glucose solution immediately after centrifuging proved of little value for maintaining motility. Although a low motility rating was maintained for three to five days in semen so treated, the motility in the untreated semen was very much better and was maintained long after the treated semen had died.

Replacing the sperm fluid with four volumes of E-Y-B dilutor immediately after centrifuging was decidedly beneficial to the spermatozoa for the

first four days of storage. The average results of such treatment of 14 samples of semen from six bulls compared with motility ratings of the same samples untreated are presented in figure 3. Here again it was noticed that very good motility was secured from spermatozoa stored in E-Y-B dilutor for four days. On the fifth day of storage, however, motility was as good in the untreated as in the treated semen; and thereafter the treated semen died rapidly while a low motility rating was maintained for some time in the natural semen. In six of the 14 samples, however, survival time was as long or longer in the treated as in the natural semen.

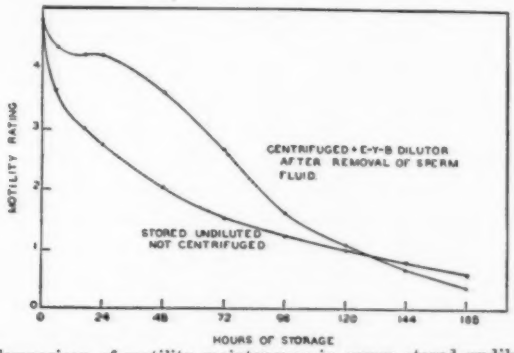

FIG. 3. Comparison of motility maintenance in semen stored undiluted and semen stored after replacing sperm fluid with egg-yolk buffer dilutor.

Since egg yolk contains practically no glucose, and since the sperm fluid containing glucose had been removed, glucose was added to three centrifuged E-Y-B diluted samples of semen with hopes of prolonging the life of the centrifuged spermatozoa. Semen so treated reacted practically the same as identical semen treated in the same way but without glucose. Hence glucose as such apparently was not the limiting factor in causing the sudden, early death of the centrifuged spermatozoa.

Insemination with stored semen. The stored semen was used for inseminations at every opportunity as it seemed that this practice should give the best test of maintenance of fertility. During the winter and spring of 1940, thirty inseminations were made with stored semen of which 15 resulted in conception. The complete record of these inseminations, including the age of the semen when used and its motility characteristics is given in table 1. These results show that the failure of certain samples of semen to produce conception was correlated with quality of the semen, irrespective of age. There was considerable variation even in samples from the same bull as to the time they could be stored successfully. The record indicates that the undiluted semen was as effective as either that diluted with E-Y-B dilutor or that centrifuged and diluted with E-Y-B dilutor. There is also indication

TABLE 1

Record of inseminations using stored semen

Bull No.	Age of semen when used (hrs.)	Motility rating at time of insemination	Maintenance of 2 motility in semen (hrs.)	Maintenance of 2 motility after use (hrs.)	Remarks
Inseminations which resulted in conception					
47	74	3	96	22	E-Y-B diluted
47	198	2	198	0	E-Y-B diluted; shipped 400 mi.
48	4	3	.....	.....	Shipped 118 mi.
48	68	1	24	0	Double insemination
48	11	3	24+	13+	Shipped 150 mi.
48	10	3	.....	.....	Shipped 150 mi.
49	48	5	72	24	Shipped 40 mi.
49	72	3	96	24	
54	44	4	144	100	
54	24	4	72	48	
40	115	3	120	5	
50	24	2	24	0	
50	4	4	24	20	Shipped 40 mi.; cow with vaginitis
50	72	2	120	48	
50	20	3	48	28	Double insemination-shy breeder
Ave.	52.5	3.0	81.6	25.5	
Insemination which failed to impregnate					
47	20	4	.....	.....	
47	125	2	196	71	E-Y-B diluted, shipped 400 mi.
55*	55	1	24	0	E-Y-B diluted
55*	48	4	48	0	E-Y-B diluted
49	24	4	96	72	Cow was shy breeder
49	72	4	96	24	Same sample as above
49	120	3	144	24	
49	56	4	48	0	E-Y-B diluted and centrifuged
49	16	4	72	56	E-Y-B diluted and centrifuged
53*	20	1	6	0	Double insemination
53*	24	1	6	0	
50	96	2	96	0	
50	4	4	24	20	Heifer with vaginitis
50	4	4	24	20	Semen shipped 40 mi.
50	24	2	24	0	
57	5	1	0	0	Semen shipped 150 mi.; bull's first service in 2 weeks.
Ave.	47.2	2.7	62.9	19.1	

* Bulls of low fertility.

that semen of poor motility at the time of insemination was ineffective, although there were exceptions. One cow was settled with semen showing a motility rating of only 1. Some samples of semen were used on two cows, one of which conceived and the other did not, so evidently the genital mechanism of the cow is quite important in affecting the use of stored semen. The

oldest semen which resulted in conception (also the oldest tried) was stored 198 hours. A live calf has now been dropped as a result of this insemination. The next oldest semen was stored 115 hours. Three samples stored over 90 hours failed to produce conception. The ratio of two services per conception for use of all the stored semen, considering all types of cows and bulls included in the study, is not excessive. There is indication that a fair conception rate should result from use of good quality semen from fertile bulls which has been stored 48 to 72 hours, provided that it shows good motility at the time of insemination.

DISCUSSION

In handling or preparing semen for storage, much care is necessary. The full initial power and energy of the spermatozoa must be preserved insofar as possible. Temperature shock should be avoided by changing the temperature gradually. The storage temperature should be reached promptly after collection, however, and it should be low. Ordinary household refrigerator temperature of 40° F. has been found satisfactory. This study has clearly shown that room temperature (70° F.) is too high for storage of bull semen. Temperature of melting ice (32° F.) was too low for best motility maintenance, although semen can be kept alive for long periods at that temperature. It may be that the cooling and warming around 32° F. are too rapid, or that temperatures so low may have a deleterious effect on the spermatozoa.

The use of diluents to aid in preserving fertility of spermatozoa was not necessary. Although the egg-yolk buffer dilutor increased the vigor of motility for the first few days of storage, it did not prolong the life of good quality semen; and in the few actual inseminations of cows in which it was tried, it was not superior to the undiluted semen in fertility. In view of this fact, the main use of diluents seems to be still one of increasing volume. In cases where an increase in volume is desired, however, the egg-yolk buffer dilutor should be used in preference to others herein reported as it was the only one which gave a favorable effect to spermatozoa motility. The short beneficial action of the egg-yolk buffer does not seem to be by way of nutrition of the spermatozoa for they died quicker in it than they did in good quality undiluted semen stored at 40° F.

Increasing the concentration of bull spermatozoa was not conducive to sperm survival. Removal of the accessory sex gland fluids by centrifuging did increase motility, however, when a favorable diluent was used for replacement. Since storage of the concentrated spermatozoa was not successful, it appears that bull spermatozoa require some medium, probably for the elimination of waste products, for best survival. The special treatment necessary to centrifuge and dilute bull semen may have valuable application

in the shipment of semen over long distances where it will be used within 48 to 72 hours. Further practical work is necessary to prove this.

CONCLUSIONS

1. Ordinary household refrigerator temperature of 40° F. was entirely satisfactory for storage of bull semen.

2. Room temperature or near freezing temperature was undesirable for storage of bull spermatozoa, although the effect of the latter was not markedly injurious.

3. Glucose solutions and Milovanov's S–G–C–2 dilutor were of no value in increasing the length of survival time in stored bull semen.

4. Egg-yolk buffer dilutor (Phillips, 11) was beneficial to motility of bull spermatozoa for the first 100 hours of storage. Semen varied in its reaction to dilution with this diluent.

5. Very vigorous motility was obtained for the first 100 hours of storage by removing the sperm fluid from the spermatozoa and diluting with egg-yolk buffer dilutor.

6. Good quality semen survived longer when stored *undiluted* than when diluted with any of the diluents tried.

7. Good quality semen which has been stored undiluted at 40° F. can be used for insemination with a reasonable degree of fertility for storage periods up to two to three days. Conception with dairy bull semen stored at longer intervals is possible, as demonstrated in this study, but the practicability of using such semen is questionable in view of the low ratio of pregnancies resulting.

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QUANTITATIVE DETERMINATION OF ALPHA AND BETA LACTOSE IN DRIED MILK AND DRIED WHEY

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INTRODUCTION

The physical properties of many milk products, particularly dried milk and whey, are greatly influenced by lactose. Therefore, it is important to know whether the lactose is in the crystalline state and, if so, whether crystallized as the alpha or the beta form and the extent to which crystallization has progressed.

The presence of alpha hydrate and beta crystals can be demonstrated qualitatively by using the seeding tests described by Sharp (2). Troy and Sharp (3) described briefly a method for determining the relative amounts of alpha and beta lactose. They did not give details because the method had not been studied sufficiently at that time. The present article gives the details of procedure and some of the supporting evidence on the points of technique involved. The basis of this method is to obtain quickly a clarified solution of the product, polarize the solution at once, allow the solution to stand until the forms of lactose reach equilibrium, and finally polarize again. The relative amounts of alpha and beta lactose are calculated from the change in rotation; the amount of total anhydrous lactose is calculated from the final rotation.

REAGENTS AND APPARATUS

Alcoholic mercuric chloride. Dissolve 264 grams of mercuric chloride in 1000 ml. of 95 per cent ethyl alcohol, hold overnight and filter. Each determination requires 10 ml.

Norrit suspension. Suspend 120 grams of norrit in 900 ml. of water, add enough N HCl to give a pH of 4–5 and then make up to 1 liter. Each determination requires 5 ml. of the freshly agitated suspension.

Previous to preparing the suspension 125 grams of norrit should be refluxed 10 hours with 1 liter of 1 per cent hydrochloric acid and washed by refluxing repeatedly with distilled water. Acid extraction is not necessary with some samples of norrit; also the alkalinity of some samples is not sufficient to require adjusting of the pH to 4–5 if heavily buffered solutions are analyzed.

Citric acid solution. A solution containing 0.2 to 0.3 per cent citric acid mono-hydrate is prepared. This solution is needed to obtain clear filtrates from badly heated or brown whey; otherwise water is used. Each analysis of dried whey requires 45 ml.

Received for publication February 25, 1941.

Oxalic acid dihydrate. Crystalline solid, about 0.02 to 0.04 gram used for each determination; required for all dried milk.

Oxalic acid solution. A solution containing 0.9 gm. oxalic acid dihydrate per liter is prepared. All dried milk samples are dissolved in 45 ml. of this solution.

Zinc chloride. Crystalline solid about 0.1–0.2 gram used for each determination on dried whey if required.

Apparatus. Balance, porcelain mortar and pestle, 100 ml. volumetric flask, erlenmeyer flask, funnel, watch glasses, fluted crepe filter paper, polariscope, stop-watch, 4 decimeter water-jacketed polariscope tube and intense light (589 mμ).

METHOD

Clarification procedure. Weigh 2.500 grams of the dried milk or whey and transfer to a dry porcelain mortar (the sample taken should contain not less than 1 gram nor more than 2.5 grams of lactose). Dry grind the sample for approximately a minute in the mortar, add 2.0 ml. of distilled water at 17° C. to a whey, or 10 ml. oxalic acid solution at 17° C. to a milk. Start a stop watch and wet grind to produce a smooth thin paste which then grind vigorously. Add more solvent and continue to grind until about 30 ml. have been added. Pour the mixture into a 100 ml. volumetric flask, into which has previously been introduced 5 ml. of norrit suspension. Rinse the mortar with additional 3 to 4 ml. portions of solvent until a total amount, 45 ml. at 17° C., has been used. (If the final filtrate obtained from a dried whey is not clear the citric acid solution should be substituted for water up to this point.) Complete the rinsing with 15–25 ml. of water at 25° C. (wash bottle) and thus transfer the entire sample with the lactose completely dissolved and extracted, to the volumetric flask. Swirl the contents. Swirling is advised to reduce foaming.

Add to the volumetric flask 10 ml. of the ethyl alcoholic solution of mercuric chloride. Rotate and swirl the contents of the flask 0.5–1.0 minute in order to mix thoroughly. Make up to the 100 ml. mark with distilled water at 25° C. When alcohol and water are mixed a slight rise in temperature results. For this reason, the temperature of the water used in extracting and making up the solution should be adjusted so that the final temperature after mixing and diluting to the mark will be approximately 25° C. The elapsed time at this stage should be 2 to 3 minutes. Shake and mix the contents of the volumetric flask for 30 seconds and pour the entire contents of the flask at once onto a fluted crepe filter paper. (This filter should not be folded too tightly or broken paper fibers will be delivered with the filtrate.) Return the filtrate to the filter until delivered clear. Then filter directly into a dry, water-jacketed polariscope tube maintained at a temperature of 25° C. Collect the remainder of the filtrate in a dry erlenmeyer flask. Keep the filter covered to minimize evaporation.

Polariscopic readings. As soon as the polariscope tube is filled a reading is taken, and additional readings are taken at minute intervals until a total of 11 has been made. Unless the temperature of the solution is 25° C. at the start the first few readings will be in error. The first reading of the polariscope is usually obtained within 5 minutes after starting the stop watch. Occasionally the time will be somewhat longer, if the solution filters slowly.

The 11 polariscopic readings are plotted against the time, as indicated by the readings of the stop watch. The best line is drawn through these points, and it is extrapolated to zero time—that is, the time of adding the water and starting of the stop watch. The readings of the polariscope extrapolated to zero time give the initial rotation, I, of the solution. The final rotation is obtained after the solution has stood at least 8 hours. It is often most convenient to add a drop of toluene and allow the solutions to stand overnight at room temperature (25° C.), in flasks. Ten readings of the final rotation are made, and the average, called F, is used in the equations given below. If a precipitate forms on standing overnight, filter the solution. Whey filtrates in which colloidal material is suspended can usually be cleared by addition of 0.1–0.2 gram of solid zinc chloride. It may be necessary to heat the solution in a water bath for a few minutes until flocculation occurs. The solution is filtered through a fine-grained filter paper.

Milk filtrates generally become turbid and cannot be clarified satisfactorily by means of zinc chloride. These are treated with 0.02–0.04 gram of solid oxalic acid dihydrate and filtered through a fine-grained filter after precipitation is complete.

Calculations. The relative amounts of anhydrous alpha lactose or of beta lactose are obtained by substituting the initial (I) and the final (F) rotations in one of the two equations (7) or (8), given below:

$$\% \text{ anhydrous alpha lactose} = \left(\frac{I}{F} - 0.635\right) 101.1 \qquad (7)$$

$$\% \text{ beta lactose} = \left(1.624 - \frac{I}{F}\right) 101.1 \qquad (8)$$

If information is required only as to the relative amounts of alpha and beta lactose present, it is not necessary to weigh the sample taken for the determination; but by taking an accurately weighed sample of 2.500 grams, the amount of lactose in the product can easily be determined from the final reading by multiplying the final reading by 18.15 if a 4 decimeter tube is used. If a saccharimeter instead of a polarimeter is used, the value must be changed to its equivalent reading on the saccharimeter scale.

SIGNIFICANCE OF DETAILS

The principle on which this method is based is simple and obvious. The difficulty arises in obtaining quickly a water-clear extract in which the

mutarotation of the sugar has not been accelerated by the protein precipitants. Most agents used for clarifying protein solutions are not satisfactory because they accelerate mutarotation, and we are thus limited to those precipitations which can be carried out in a neutral (pH 4–6) solution. A great number of protein precipitates were investigated both singly and in combination. An alcoholic solution of mercuric chloride was found to be the most satisfactory.

Solution of the sample. If the lactose is not dissolved before adding the alcohol, the rate of solution is generally so retarded by the alcohol as to delay the determination of the initial rotation; or some of the lactose may actually be filtered out if sufficient time is not allowed for solution. Relatively large lactose crystals are present in some samples, and solution is not sufficiently rapid unless the sample is dry ground in the mortar before the water is added.

Adding the water to the sample in the mortar permits the immediate disintegration of the lumps which form in many samples and enables the extraction to be made quickly and completely.

The extraction in the mortar is made at about 17° C. so that the heat liberated when the alcoholic mercuric chloride is added later will bring the temperature to 25° C. Unless the temperature is 25° C. at the time of the first polariscopic reading, the first few values will be in error; for the differences in refractive index produced by the difference in temperature of portions of liquid in the polariscope tube will make matching of the fields difficult.

The amount of water used at the various stages has been carefully worked out to permit the complete solution of the lactose and transfer of the sample to the 100 ml. volumetric flask, and to obtain a temperature of 25° C. in the final mixture.

Alcoholic mercuric chloride precipitation. Alcohol was used because it is a good solvent for mercuric chloride and because it also aids in the protein precipitation and prevents adsorption of lactose by the norrit. Norrit was required to remove the yellow color produced by the riboflavin and the brown color of the milk and whey produced by heat or aging. The amount of norrit recommended sufficed to decolorize all but abnormally brown (overheated or very old) samples. The slight residual yellow color of some filtrates obtained from such samples did not seriously reduce visibility in the polariscope.

Turbidity in a filtrate, slight enough to be almost imperceptible when viewed in a flask, becomes disturbing when viewed through a 4 dm. polariscope tube. A light of high intensity is recommended for fine work. We used a monochromator adjusted with calibrated quartz plates.

Varying concentrations of mercuric chloride in the alcohol, varying amounts of alcohol and varying amounts of norrit were tried and a combina-

tion of the three was worked out which gave the best results. If too much alcohol is used the solubility of the lactose is affected and also its specific rotation; yet sufficient alcohol should be used to aid in the precipitation and prevent adsorption of lactose by the norrit.

Norrit. The norrit is added in the form of a water suspension because the air contained in the dry material causes marked foaming, whereas in the suspension the air has been eliminated from the surface of the norrit.

A trace of lactose seems to adsorb on the norrit even in the presence of alcohol. Irregular behavior was occasionally observed when unextracted norrit was used. No correction is applied for the volume of the norrit (0.4 ml.) or of the protein precipitate. This is because the corrections are small, and because the amount of lactose adsorbed and the effect of the alcoholic mercuric chloride on the rotation of the lactose approximately compensate for the concentrating effect of the volume displaced by the solid phase.

To retard mutarotation, it is advisable to adjust the acidity of the norrit suspension to pH 4–5 with hydrochloric acid, and such adjustment is necessary when pure unbuffered lactose solutions are analyzed. In the analysis of dried milk and dried whey, if the highest accuracy is not desired, the extraction with hydrochloric acid and the adjustment of the pH of the norrit suspension can be dispensed with. The unextracted norrit suspensions tested had pH values of 8 to 9. The buffer value and pH of the dried milk are sufficient to reduce the pH and prevent marked acceleration of mutarotation by the alkaline norrit.

Clouding of filtrates and development of cloudiness: Most dried milks and some dried wheys, if dissolved in water, give turbid filtrates after precipitation with alcoholic mercuric chloride. A number of substances were added in the attempt to obtain clear filtrates and at the same time not interfere with the determination.

The best results were obtained with dried milks by dissolving them in the oxalic acid solution recommended. This served to delay the appearance of turbidity. Its mode of action is not clear. If the turbidity is due to a colloidal calcium compound, the oxalic acid might either delay its formation, or partially precipitate it.

Some dark samples of dried whey must be extracted with the citric acid solution as a solvent in place of water in order to obtain clear filtrates for determining the initial rotation.

Precipitation occurs in many whey filtrates and most milk filtrates during equilibration.

The turbidity of whey filtrates is usually removed by filtering through a fine paper; milk filtrates generally remain turbid. Turbidity in whey filtrates which is not removed by simple filtration can be precipitated by treatment with solid zinc chloride (0.1 – 0.2 gm.); in milk filtrates by treat-

ment with solid oxalic acid dihydrate (0.04 gm.). Warming accelerates flocculation. The flocculated material is removed with a fine filter.

Adding alkalies to accelerate final rotation. Many investigators recommend the addition of alkalies so that the final rotation can be obtained almost immediately after obtaining the initial rotation. Sodium carbonate, sodium bicarbonate and ammonium hydroxide have been recommended. This procedure, unless tested with the specific product, may lead to errors. If too much alkali is added the optical rotation of the sugar is affected. If not enough alkali is added, the mutarotation may not be accelerated as expected. Correct final rotations are obtained by acceleration with alkali only when the solution is adjusted to the proper pH. The addition of the same amount of alkali to different samples will not give the same pH because of the different buffer values, particularly in the case of dried wheys. Moreover, the addition of alkali precipitates mercuric oxide in the filtrates here employed. For these reasons it is much safer to allow the solution to reach equilibrium by standing rather than to attempt to accelerate mutarotation by alkali.

Derivation of equations. Several values are given in the literature for the specific rotations of the two forms of lactose and of their equilibrium mixture for the sodium D lines at 20° C. (1). Direct determinations at 25° C. are not reported. The specific rotations obtained in this laboratory at 25° C. with light of 589 mμ using products of high purity are as follows: anhydrous alpha lactose (weighed as the hydrate), + 89.5° beta lactose, + 35.0°; equilibrium mixture at concentrations between 10 and 250 gm. anhydrous lactose per liter, + 55.1°. All three constants apply to aqueous solutions.

Suppose A_o per cent of the lactose in a sample were in the alpha form, then 100–A_o represents the per cent in the beta form. The initial specific rotation, I_s, of a solution obtained from this sample would be given by

$$89.5A_o + 35\ (100-A_o) = 100\ I_s \qquad (1)$$

where the constants are the specific rotations of alpha and beta lactose respectively.

On equilibration at 25° C. the solution would assume a final specific rotation, F_s, and in this solution A_∞ per cent of the lactose would be in the alpha form, 100–A_∞ per cent in the beta form. For this solution

$$89.5A_\infty + 35\ (100-A_\infty) = 100\ F_s \qquad (2)$$

Dividing (1) by (2) and collecting terms gives,

$$\frac{I_s}{F_s} = \frac{54.5A_o + 3500}{54.5A_\infty + 3500} \qquad (3)$$

In the equilibrium solution at 25° C., however, we have:

$$89.5A_\infty + 35\ (100-A_\infty) = 100 \times 55.1 \qquad (4)$$

where 55.1 is the specific rotation of this solution. Solving (4) for A_∞ we obtain 36.9 and substituting this value in (3) and solving for A_o:

$$A_o = \left(\frac{I_s}{F_s} - 0.635\right) 101.1 = \% \text{ alpha} \quad (5)$$

and

$$(1-A_o) = \left(1.624 - \frac{I_s}{F_s}\right) 101.1 = \% \text{ beta} \quad (6)$$

Since the observed initial and final rotations, I and F, are made with the same solution in the same polariscope tube they are proportional to I_s and F_s and can be substituted in (5) and (6) to give (7) and (8).

$$A_o = \left(\frac{I}{F} - 0.635\right) 101.1 = \% \text{ alpha} \quad (7)$$

$$(1-A_o) = \left(1.624 - \frac{I}{F}\right) 101.1 = \% \text{ beta} \quad (8)$$

These percentages, of course, refer to total amount of anhydrous lactose in the sample as 100.

Accuracy of the method. Tables 1 and 2 show results obtained when the method was applied to the pure alpha and beta forms of lactose respectively. Inspection of table 1 shows that filtration and such possible impurities in the alcohol as acetaldehyde and acetic acid do not influence the results. Mercuric chloride and alcohol, individually and combined, so affect the rotation as to give apparent percentages of total lactose slightly below 100, and apparent percentages of alpha lactose slightly above 100.

Norrit adsorbs appreciable amounts of lactose from aqueous solution but only a very limited amount from solutions containing alcohol.

The effects of norrit, alcohol, mercuric chloride and combinations of these reagents on beta lactose solutions are shown in table 2. The results are similar to those obtained with solutions of alpha lactose. In the presence of alcoholic $HgCl_2$ the apparent percentage of total lactose is a little low, but the percentage of beta lactose is as nearly correct as the errors in the method would justify. Adsorption of beta lactose on the carbon is shown and the adsorption is also inhibited by alcohol.

Preferential adsorption of alpha lactose on norrit. Not only does norrit adsorb appreciable amounts of lactose from an aqueous solution but it adsorbs the alpha modification preferentially. This can be demonstrated clearly when appreciable amounts of both the alpha and beta forms are present in the solution, and when enough norrit is added to adsorb about one half of the lactose present. A dry mixture containing 40 per cent by weight of alpha lactose (calculated on the anhydrous basis) and 60 per cent of beta lactose was prepared. Aliquots containing 0.74 gram anhydrous lactose were dissolved and made up to a final volume of 100 ml., using variations in procedure which would demonstrate adsorption of lactose by norrit and elution by alcohol. The results are presented in table 3.

Experiments A, B, C and D show no appreciable effect of filtration and only a slight effect of 20 per cent alcohol on the ratio and rotations of the

TABLE 1

Analysis of alpha lactose hydrate, sugar weighed on anhydrous basis

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
Water and lactose alone	8.96	5.511	1.626	100.2	−0.2	100.0
	8.96	5.508	1.627	100.3	−0.3	100.0
	8.93	5.504	1.622	99.8	0.2	99.9
	8.94	5.508	1.623	99.9	0.1	100.0
Water and lactose alone, filtered	8.96	5.488	1.633	100.9	−0.9	99.6
	8.93	5.482	1.629	100.5	−0.5	99.5
	8.95	5.510	1.624	100.0	0.0	100.0
	8.92	5.514	1.618	99.4	0.6	100.1
In 4.5% alcohol	8.95	5.492	1.630	100.6	−0.6	99.7
In 9 % alcohol	8.94	5.479	1.632	100.8	−0.8	99.4
In 18 % alcohol	8.87	5.449	1.628	100.9	−0.9	98.9
In 4.5% alcohol, filtered	8.96	5.489	1.632	100.8	−0.8	99.6
In 9 % alcohol, filtered	8.94	5.479	1.632	100.8	−0.8	99.4
In 18 % alcohol, filtered	8.81	5.437	1.620	99.6	0.4	99.4
In 1.2% $HgCl_2$	8.98	5.480	1.639	101.5	−1.5	99.5
In 2.3% $HgCl_2$	8.94	5.472	1.634	101.0	−1.0	99.3
In 4.5% $HgCl_2$	8.94	5.474	1.633	100.9	−0.9	99.4
In 1.2% $HgCl_2$, filtered	8.98	5.464	1.643	101.9	−1.9	99.2
In 2.3% $HgCl_2$, filtered	8.97	5.475	1.638	101.4	−1.4	99.4
In 4.5% $HgCl_2$, filtered	8.91	5.466	1.630	100.6	−0.6	99.2

TABLE 1—(*Continued*)

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
+ 10 ml. 26.4% alcoholic $HgCl_2$	8.85	5.457	1.622	99.8	0.2	99.0
	8.88	5.445	1.631	100.7	− 0.7	98.8
+ 10 ml. 26.4% alcoholic $HgCl_2$, filtered	8.90	5.473	1.626	100.2	− 0.2	99.3
	8.91	5.484	1.625	100.1	− 0.1	99.5
	8.92	5.479	1.628	100.4	− 0.4	99.4
+ 20 ml. 26.4% alcoholic $HgCl_2$, filtered	8.85	5.431	1.630	100.6	− 0.6	98.6
+ 10 ml. 26.4% alcoholic $HgCl_2$ containing .75% acetaldehyde	8.92	5.466	1.632	100.8	− 0.8	99.2
+ 10 ml. 26.4% alcoholic $HgCl_2$ containing .25% acetic acid	8.95	5.467	1.637	101.3	− 1.3	99.2
+ .60 gm. norrit in suspension	8.52	5.236	1.627	100.3	− 0.3	95.0
	8.56	5.257	1.628	100.4	− 0.4	95.4
+ 10 ml. 26.4% alcoholic $HgCl_2$ + .60 gm. norrit in suspension	8.85	5.450	1.624	100.0	0.0	98.9
	8.85	5.449	1.624	100.0	0.0	98.9
	8.82	5.451	1.618	99.4	0.6	98.9
	8.85	5.451	1.624	100.0	0.0	98.9

TABLE 2

Analysis of beta lactose

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
Water and lactose alone	3.51	5.514	.637	0.2	99.8	100.1
	3.48	5.492	.634	−0.1	100.1	99.7
Water and lactose alone, filtered	3.50	5.529	.633	−0.2	100.2	100.4
	3.52	5.527	.637	0.2	99.8	100.3
+ 10 ml. 26.4% alcoholic $HgCl_2$	3.49	5.489	.636	0.1	99.9	99.6
	3.46	5.476	.632	−0.3	100.3	99.4
+ 10 ml. 26.4% alcoholic $HgCl_2$ filtered	3.47	5.476	.634	−0.1	100.1	99.4
	3.48	5.475	.636	0.1	99.9	99.4
+ .60 gm. norrit in suspension	3.36	5.249	.640	0.5	99.5	95.3
	3.33	5.257	.633	−0.2	100.2	95.4
+ .60 gm. norrit in suspension	3.54	5.467	.648	1.3	98.7	99.2
	3.51	5.485	.640	0.5	99.5	99.6
+ 10 ml. 26.4% alcoholic $HgCl_2$	3.47	5.494	.632	−0.3	100.3	99.7
	3.47	5.478	.633	−0.2	100.2	99.4

forms of lactose. In experiment E about half of the lactose was adsorbed on the norrit and the proportion of the two forms remaining unadsorbed was altered from that of the original solution, as shown by the decrease in the ratio $\frac{I}{F}$. If the forms were adsorbed in proportion to their concentration in the original solution, then the filtrate would be relatively richer in alpha lactose, but actually it is poorer. Furthermore, experiment F showed that when the adsorbed sugar was eluted from the norrit by alcohol the eluate was richer in alpha as shown by the increase in the ratio $\frac{I}{F}$. This demonstrates the preferential adsorption of the alpha form by norrit. Data obtained in a series of other experiments not recorded here can be explained on this basis.

In experiment G lactose in solution was added to a mixture of norrit suspension and alcohol. Practically no adsorption took place and the ratio of the two forms in the filtrate remained unchanged. The previous adsorption of lactose by the norrit does not alter the ratio of alpha to beta in the filtrate when the norrit surface is later freed of lactose by the addition of alcohol as the last step in preparing the mixture. This was demonstrated in experiment H. Experiments A, B, C, D, G, and H show good agreement with the calculated values, for the rotations and also the proportions of alpha and beta lactose in the mixture.

Recovery of lactose added to dried milk and dried whey. Table 4 shows the percentage recovery of specific forms of lactose when added to dried milk

TABLE 3

Adsorption of lactose by norrit, preferential adsorption of the alpha form and elution with alcohol

The suspension of extracted norrit added, contained 2.2 grams. In each experiment of 0.74 gram of anhydrous lactose was made up to 100 ml. and the solutions were polarized at 25° C. in a 4 decimeter tube.

Expt.	Preparation of the solutions	Rotations			Lactose present as		Total lactose in sample %
		I	F	$\frac{I}{F}$	Alpha %	Beta %	
A	Dissolved in water.	1.679	1.620	1.036	40.5	59.5	99.3
		1.680	1.616	1.040	40.9	59.1	99.1
B	Dissolved in water, filtered.	1.672	1.617	1.034	40.3	59.7	99.1
		1.675	1.623	1.032	40.1	59.9	99.5
C	Dissolved in H_2O; sol'n. added to alcohol + H_2O; contained 20% alcohol after diluting to mark.	1.648	1.610	1.024	39.3	60.7	98.7
		1.643	1.612	1.019	38.8	61.2	99.8
D	Dissolved in H_2O; sol'n. added to alcohol + H_2O; contained 20% alcohol after diluting to mark; filtered.	1.655	1.610	1.028	39.7	60.3	98.7
		1.635	1.593	1.026	39.5	60.5	97.7
E	Dissolved in H_2O; sol'n. added to norrit, brought to volume, centrifuged, decanted, filtered.	0.842	0.858	0.981	35.0	65.0	52.6
		0.854	0.862	0.991	36.0	64.0	52.8
F	Packed norrit from above extracted with 20% alcohol.	0.760	0.715	1.063	43.3	56.7	43.8
		0.829	0.767	1.081	45.1	54.9	47.0
G	Aqueous sol'n. of sugar added to norrit + alcohol; sol'n. contained 20% alcohol diluting to mark.	1.662	1.606	1.035	40.4	59.6	98.5
		1.674	1.623	1.031	40.0	60.0	99.5
H	Aqueous sol'n. of sugar added to norrit for adsorption; sugar eluted by adding alcohol before diluting to mark; filtrate contained 20% alcohol.	1.658	1.612	1.029	39.8	60.2	98.8
		1.642	1.599	1.027	39.6	60.4	98.0
I	Mixture as made by weighing, calculated.	1.681	1.631	1.031	40.0	60.0	100.0

TABLE 4

Recovery of added lactose

Lactose added: 1 gm. pure anhydrous Alpha lactose (weighed as hydrate) or
1 gm. pure anhydrous Beta lactose.

Sample analyzed	Lactose added	Rotations, 4 dm. tube 25° C.			Amount of lactose		Total lactose in product %	Gm. anhydrous lactose in filtrate			Per cent added lactose recovered		
		I	F	$\frac{I}{F}$	As Alpha %	As Beta %		Total	Alpha	Beta	Total	Alpha	Beta
Skimmilk 40 (glass)	None	2.75	2.609	1.054	42.4	57.6	47.4	1.1837	0.5019	0.6818	.....	.....	.....
	Alpha	6.37	4.847	1.314	68.6	31.4	.....	2.1991	1.5086	0.6905	101.5	100.7	.....
	Beta	4.21	4.874	0.864	23.2	76.8	.....	2.2114	0.5130	1.6984	102.8	.....	101.7
Whey 1 (Alpha)	None	5.96	3.818	1.561	93.6	6.4	69.3	1.7322	1.6213	0.1109	.....	.....	.....
	Alpha	9.57	6.028	1.588	96.3	3.7	.....	2.7349	2.6337	0.1012	100.3	101.2	.....
	Beta	7.46	6.084	1.226	59.8	40.2	.....	2.7603	1.6507	1.1096	102.8	.....	99.9
Whey 2 (Beta)	None	2.57	3.595	0.715	8.1	91.9	65.2	1.6311	0.1321	1.4990	.....	.....	.....
	Alpha	6.19	5.827	1.062	43.2	56.8	.....	2.6437	1.1421	1.5016	101.3	101.0	.....
	Beta	4.03	5.822	0.692	5.8	94.2	.....	2.6414	0.1532	2.4882	101.0	.....	98.9
Whey 67 (Alpha)	None	5.51	3.470	1.588	96.3	3.7	63.0	1.5743	1.5161	0.0582	.....	.....	.....
	Alpha	9.01	5.647	1.596	97.2	2.8	.....	2.5621	2.4904	0.0717	98.8	97.4	.....
	Beta	6.96	5.667	1.228	60.0	40.0	.....	2.5711	1.5427	1.0284	99.7	.....	97.0

TABLE 5

Amount of alpha and beta lactose in selected samples of dried whey and milk

Sample No. and nature	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total anhydrous lactose in product %
	I	F	$\frac{I}{F}$	% As Alpha	As Beta %	
51. Whey, beta type	2.58	3.45	0.748	11.4	88.6	62.6
	2.55	3.47	0.735	10.1	89.9	63.0
53. Whey, beta type	3.31	3.84	0.862	22.9	77.1	69.7
	3.27	3.96	0.826	19.3	80.7	71.9
71. Whey, beta type	3.00	3.64	0.824	20.1	79.9	66.1
	2.97	3.67	0.809	17.6	82.4	66.7
58. Whey, beta type	3.37	3.70	0.911	27.9	72.1	67.2
	3.35	3.76	0.891	25.9	74.1	68.2
64. Whey, beta type	3.19	3.63	0.879	24.7	75.3	65.9
	3.22	3.65	0.882	25.0	75.0	66.2
F. Whey, beta type	3.07	3.73	0.823	19.0	81.0	67.7
	3.13	3.74	0.837	20.4	79.6	67.9
82. Modified whey	2.27	2.20	1.032	40.1	59.9	39.9
	2.26	2.20	1.027	39.6	60.4	39.9
24. Whey, alpha type, pan condensed	5.40	3.56	1.517	89.2	10.8	64.6
	5.47	3.58	1.528	90.3	9.7	65.0
40S. Whey, alpha type, pan condensed	6.13	3.88	1.580	95.5	4.5	70.4
41S. Whey, alpha type, pan condensed	5.81	3.74	1.553	92.8	7.2	67.9
67. Whey, alpha type, pan condensed (old analysis)	5.62	3.63	1.548	92.3	7.7	65.9
	5.60	3.62	1.547	92.2	7.8	65.7
65. Whey, alpha type, spray dried	5.09	3.48	1.463	83.7	16.3	63.2
	5.13	3.51	1.462	83.6	16.4	63.7
72. Whey, alpha type, spray dried	5.18	3.43	1.510	88.5	11.5	62.3
	5.24	3.48	1.506	88.1	11.9	63.2
10. Skimmilk, roll dried	2.70	2.56	1.055	42.5	57.5	46.5
11. Skimmilk, roll dried	2.12	2.02	1.050	42.0	58.0	.....
M20. Skimmilk, roll dried	2.92	2.75	1.062	43.2	56.8	49.9
M40. Skimmilk, roll dried	2.76	2.60	1.062	43.2	56.8	47.2
M60. Skimmilk, roll dried	2.69	2.55	1.055	42.5	57.5	46.3
M80. Skimmilk, roll dried	2.51	2.43	1.033	40.2	59.8	44.1

or whey samples. The recovery of total lactose of each individual form is as good as might be expected. The data in table 4 show an increase in experimental error, when the method is applied to dried milk or whey—as compared to the errors seen in tables 1 and 2 where pure sugars were analyzed. Several additional potential sources of error are introduced in analyzing dried milk and whey.

Alpha and beta lactose in samples of dried milk and whey. Table 5 presents analyses of various typical dried products and shows the wide variations encountered in the relative amounts of alpha and beta lactose, ranging from about 90 per cent in the beta to 95 per cent in the alpha form. The amount of total lactose in the products also showed considerable variation.

SUMMARY

1. A method is described for the determination of the amounts of alpha and beta lactose in dried milk and whey, and also of the total amount of anhydrous lactose in these products.

2. A clear, colorless extract of the product is obtained quickly, using an alcoholic solution of $HgCl_2$ as protein precipitant, and norrit as a decolorizing agent. The filtrate is polarized as soon as possible and again after standing. The relative amounts of alpha and beta lactose are computed from the change in optical rotation. Total lactose is calculated from the final (equilibrium) rotation and sample weight.

3. Alpha and beta lactose gave satisfactory results when analyzed by this method, as did also dried milk and whey, alone and with additions of alpha and beta lactose.

4. The composition of the lactose in dried whey was shown to range from 90 per cent beta to 95 per cent alpha, on the anhydrous basis.

5. Norrit adsorbs lactose from aqueous solution, the alpha form being adsorbed preferentially. Lactose is eluted from the norrit by 10 to 20 per cent alcohol.

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FACTORS AFFECTING THE ACTIVITY AND HEAT RESISTANCE OF SWISS CHEESE STARTER CULTURES. III. EFFECT OF VARIATIONS IN TIME AND TEMPERATURE OF INCUBATION AND OF STORAGE ON ACTIVITY OF CULTURES*

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The variation permissible in methods of preparation and storage of cultures of lactic acid bacteria without causing reduction in their activity remains a practical problem to the dairy manufacturer. In a previous paper Elliker and Frazier (1) reported the influence of incubation temperature on heat resistance of certain starter organisms for Swiss cheese; in their experiments, incubation periods common in actual practice were employed. The present study was undertaken to ascertain how much variation in time and temperature of incubation was permissible without loss in activity of the starter organisms. The effect of these same variations on heat resistance of cultures will be discussed in the following paper of this series.

A definite relationship between the titratable acidity of starter cultures grown at a constant temperature and the quality of Swiss cheese was observed by Frazier and associates (3). The most effective milk starter cultures of *Lactobacillus helveticus*, Strain 39a, had titratable acidities of 1.00 to 1.09 per cent (as lactic acid) after growth at 37.5° to 39° C. for 12 hours. In experiments with cultures of *Streptococcus thermophilus*, Strain C–3, best results were noted when milk cultures showed titratable acidities of 0.70 to 0.75 per cent after 12 hours at 37° C. Thus the preparation of starter cultures requires controlled temperatures and periods of incubation for production of the desired rate of lactic acid fermentation in the cheese in the press. Information bearing on the methods or procedures of preparing active starter cultures should be helpful not only to the makers of Swiss cheese but also to all who handle active cultures of thermoduric lactic acid bacteria.

EXPERIMENTAL

Pure cultures of *Lactobacillus helveticus*, Strain 39aW, and *Streptococcus thermophilus*, Strain C–3, were grown at constant temperatures in freshly autoclaved ten per cent reconstituted skimmilk. After seven to nine consecutive transfers at a given temperature, the activity of each mother culture, as evidenced by amount of growth and fermentation, was de-

Received for publication March 10, 1941.

* This work was supported by a grant from the Wisconsin Alumni Research Foundation and is published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

termined. For bulk culture, an inoculum of 0.25 per cent, from a mixture of equal parts of mother culture and a two per cent solution of sodium citrate, was transferred into 400 ml. of fresh skimmilk medium. Bulk cultures were incubated for 12 hours, and usually at the same temperature as that previously used for the mother culture. The amount of growth in bulk cultures was determined by direct microscopic count of living cells, according to the method of Frazier and Boyer (2). Acidity of bulk cultures was measured by titration, and pH was calculated from the potentials shown by the quinhydrone electrode. Direct counts were made also of mother cultures after incubation; in some studies, the acidities of mother cultures were used for comparison. Representative experiments are summarized briefly.

Influence of incubation time of mother cultures grown at 37° C. on the activity of 12-hour bulk cultures. In a series of several experiments the cultures were incubated at 37° C. and transferred every 12, 24, 36, 48 and 168 hours for seven to nine successive transfers. The recommended period for incubation of Swiss cheese starters has been 12 to 14 hours at 37° C., so that their maturity could be judged by the acidity produced in that time. A comparison of activity of 12-hour bulk cultures prepared from mother cultures incubated at 37° for periods longer than those commonly employed is made in table 1; arithmetic averages of several experiments are given.

TABLE 1

Differences in activity of bulk cultures after 12 hours at 37° C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 37° C. and transferred every 12, 24, 36, 48 or 168 hours*

Mother culture		Activity of bulk culture		
Incubation time	Direct count	Direct count	Titratable acidity	pH
hours	*millions/ml.*	*millions/ml.*	*per cent*	
		L. helveticus		
12	1,013	1,294	1.05	4.10
24	1,041	1,249	1.04	4.18
36	796	703	0.77	4.42
48	714	464	0.63	4.70
168	519	2	0.26	5.90
		Str. thermophilus		
12	1,032	1,079	0.82	4.41
24	1,017	1,363	0.81	4.46
36	797	597	0.73	4.51
48	732	380	0.63	4.67
168	410	19	0.27	5.88

* Seven to nine successive transfers.

Cultures of *L. helveticus* or *Str. thermophilus* after numerous transfers at 37° C. every 12 or 24 hours were similar in growth and fermentation in 12-

hour bulk cultures. Periods of incubation greater than 24 hours, however, resulted in a decrease in the numbers of cells in both mother cultures and 12-hour bulk cultures, while the acidities developed in bulk cultures decreased progressively as the age of mother culture increased.

Influence of incubation time of mother cultures grown at 40°, 42°, or 45° C. on the activity of 12-hour bulk cultures. Since relatively short periods (12 to 24 hours) of incubation of mother cultures grown at 37° C. resulted in no marked change in the activity of 12-hour bulk cultures, higher temperatures of incubation for similar periods were employed. It might be expected that the incubation of mother cultures at temperatures above 37° and for periods greater than 24 hours would seriously decrease acid production in 12-hour bulk culture. The results of preliminary experiments substantiated this belief.

TABLE 2

Differences in activity of bulk cultures after 12 hours at 40°, 42°, or 45° C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 40°, 42°, or 45° C. and transferred every 12 and 24 hours

Mother culture		Activity of bulk culture			Incubation temperature of all cultures
Incubation time	Direct count	Direct count	Titratable acidity	pH	
hours	*millions/ml.*	*millions/ml.*	*per cent*		*°C.*
		L. helveticus			
12	880	1,070	1.08	4.01	40
24	871	967	0.99	4.21	40
12	1,579	1,309	0.97	4.10	42
24	864	1,028	0.93	4.19	42
12	.....	.....	0.46	5.13	45
24	.....	.....	0.68	4.58	45
		Str. thermophilus			
12	739	828	0.70	4.63	40
24	634	733	0.70	4.64	40
12	1,348	1,211	0.76	4.35	42
24	1,444	923	0.74	4.38	42
12	.....	.....	0.70	4.66	45
24	.....	.....	0.67	4.65	45

The effect of cultivation of mother cultures at 40°, 42°, and 45° C. for successive 12- and 24-hour periods upon the activity of 12-hour bulk cultures is shown in table 2. Mother cultures of *L. helveticus* grown at 40° or 42° C. for 12 hours were slightly more active in 12-hour bulk culture than the 24-hour mother cultures; however, incubation at 45° gave irregular data, apparently due to the close approach of the incubation temperature to the maximum temperature of growth of this organism. A comparison of the data on the bulk cultures prepared from 12- and 24-hour mother cultures of *Str. thermophilus* provides no conclusive evidence that the 12-hour mother

cultures at a given temperature of incubation are superior in fermentation or growth to the 24-hour mother cultures carried at the same temperature.

Differences in mother cultures as shown by the activity of bulk cultures in the initial phases of the growth cycle. Although differences in acidity and total cell counts could not be demonstrated in 12-hour bulk cultures, the possibility that differences in growth and fermentation existed during the first few hours after inoculation was investigated. A comparison of bulk cultures, made 2.5 to 3 hours after inoculation, appears in table 3.

TABLE 3

Comparison of amount of growth and fermentation of bulk cultures shortly after inoculation with mother cultures which had been transferred at different temperatures every 12 and 24 hours

Mother culture		Activity of bulk culture				
Incubation		Incubation		Direct count of inoculum	Direct count after incubation	Drop in pH
Time	Temp.	Temp.	Time			
hours	*°C.*	*°C.*	*hours*	*millions/ml.*	*millions/ml.*	
L. helveticus						
12	37	37	2.5	1.82	8.42	0.06
24	37	37	2.5	1.60	19.1	0.07
12	37	42	2.5	1.33	16.6	0.13
24	37	42	2.5	1.08	12.7	0.11
12	40	40	3.0	2.02	31.9	0.22
24	40	40	3.0	2.21	15.9	0.04
12	42	42	3.0	.....	.....	0.07
24	42	42	3.0	.....	.....	0.02
Str. thermophilus						
12	37	37	2.5	.810	109.	0.26
24	37	37	2.5	.916	77.0	0.20
12	40	40	2.5	.410	45.0	0.34
24	40	40	2.5	.430	34.7	0.16
12	40	43	2.5	.410	77.4	0.46
24	40	43	2.5	.430	59.9	0.27
12	42	42	2.5	.....	.....	0.44
24	42	42	2.5	.....	.....	0.22

Mother cultures of *L. helveticus*, incubated at 37° for 12 and 24 hours, were equally as active in 2.5-hour bulk cultures at 37° as in similar subcultures at 42°. Cultures of *Str. thermophilus* handled in a similar manner were shown to be equivalent in activity when mother cultures were grown at 37° for 12 or 24 hours. Cultures of both species of bacteria grown above 37° for 12 or 24 hours exhibited greatest variations in activity when the amounts of decrease in pH were compared. The differences in activity of cultures observed during the early hours of incubation were absent at the time 12-hour bulk cultures were compared. The capacity of 24-hour cultures to overtake the 12-hour mother cultures was more apparent with *Str. thermophilus* than *L. helveticus*. It may be related to the increased fer-

menting capacity of old cells observed by Rahn *et al.* (6) with old cells of *Str. lactis* beginning to multiply in a fresh medium.

Influence of low storage temperatures after the incubation of mother cultures upon the activity of 12-hour bulk cultures. The high degree of activity exhibited by mother cultures transferred at consecutive 24-hour intervals suggested that relatively short periods of incubation near the optimum temperature of growth for these bacteria could be used, followed by storage at temperatures below the optimum for growth. Mother cultures were incubated at 37° for 9, 12, or 16 hours, placed promptly at 0°, 20°, and 30° C., respectively, and stored until each culture was 24 hours old. The activities of bulk cultures grown at 37° for 12 hours are compared in table 4. Mother cultures of *L. helveticus* were stored at 0° and 20° until 24 hours old, following an initial incubation period of 12 to 16 hours at 37°, without reduction in growth or fermentation. Storage at 30°, however, resulted in decreased titratable acidities of the bulk cultures. Although Kopeloff *et al.* (4) reported that storage of other lactobacilli at 20° was less harmful than at lower temperatures, the data shown here indicate no marked differences in viability of *L. helveticus* during storage at 0° or 20° C.

TABLE 4

Differences in activity of bulk cultures after 12 hours at 37°C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 37°C. for 9, 12 or 16 hours and then stored at 0°, 20°, or 30°C. until 24 hours old

Mother cultures					Activity of bulk cultures		
Incubation time at 37°C.	Storage		pH	Direct count	Direct count	pH	Titratable acidity
	Time	Temp.					
hours	*hours*	*°C.*		*millions/ml.*	*millions/ml.*		*per cent*
L. helveticus							
9	15	0	4.55	674	1,069	4.54	0.76
12	12	0	4.23	727	863	4.47	0.85
16	8	0	4.09	739	876	4.45	0.85
12	12	20	4.21	887	790	4.47	0.87
16	8	20	4.14	1,015	879	4.42	0.87
12	12	30	4.02	623	———	4.50	0.75
16	8	30	3.93	830	———	4.47	0.75
Str. thermophilus							
9	15	0	4.78	982	744	4.56	0.70
12	12	0	4.51	892	594	4.58	0.71
16	8	0	4.39	792	904	4.57	0.71
12	12	20	4.46	927	1,299	4.67	0.67
16	8	20	4.41	1,156	576	4.65	0.68
12	12	30	4.36	1,336	1,014	4.55	0.71
16	8	30	4.33	744	746	4.55	0.71

Cultures of *Str. thermophilus*, when treated in a manner similar to that

described for *L. helveticus*, were equally as active in bulk culture after storage at 0° and 20° C. as they were after being held at 30°. The initial incubation time of mother cultures before storage, whether 9, 12, or 16 hours, had no marked influence on the direct counts or acidities of the bulk cultures. Results of experiments (5) analogous to the one just described, except that bulk cultures were incubated at 30°, confirmed these observations and revealed that *L. helveticus* and *Str. thermophilus* could be stored at 0°, 10° or 20° C. for similar periods without reduction in activity.

Since a short storage period was harmless to either *L. helveticus* or *Str. thermophilus*, the effect of variations in storage time on growth and fermentation of mother cultures was studied. Cultures held at 42° for 12, 16, or 24 hours were continued at 20° for different periods. According to results shown in table 5, the incubation and storage time of mother cultures of *L. helveticus* and *Str. thermophilus* could be varied considerably without altering significantly the numbers of cells and titratable acidities of 12-hour bulk cultures. Cultures incubated at 42° for 12 or 24 hours could be stored

TABLE 5

Differences in activity of bulk cultures after 12 hours at 42°C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 42°C. for 12, 16, or 24 hours and then stored at 20°C. until 24, 48 or 96 hours old

Mother culture				Activity of bulk culture		
Incubation time at 42°C.	Storage at 20°C.	pH	Direct count	Direct count	pH	Titratable acidity
hours	*hours*		*millions/ml.*	*millions/ml.*		*per cent*
L. helveticus						
12	12	4.15	516	965	4.27	0.95
16	8	4.00	659	766	4.25	0.93
12	36	3.89	893	1,013	4.27	0.98
12	84	3.78	784	1,006	4.14	1.01
24	24	3.71	886	889	4.35	0.93
24	72	3.69	601	641	4.30	0.93
12	none	.....	1,579	1,309	4.10	0.97
24	none	.....	864	1,028	4.19	0.93
Str. thermophilus						
12	12	4.47	541	856	4.44	0.75
16	8	4.39	592	505	4.55	0.71
12	36	4.29	493	1,135	4.64	0.61
12	84	4.24	807	1,049	4.44	0.74
24	24	4.20	462	1,029	4.67	0.64
24	72	4.06	849	881	4.40	0.72
12	none	.....	1,348	1,211	4.35	0.76
24	none	.....	1,444	923	4.38	0.74

at 20° an additional 84 or 72 hours, respectively, without reduction in their activity. Storage under these conditions produced mother cultures equiva-

lent in bulk culture activity to cultures transferred every 12 or 24 hours near the optimum growth temperature. During storage at 20° a substantial number of cells die off, and mother cultures increase slowly in acidity. At this temperature, however, the effect of the developed acidity on the enzyme system of the majority of cells is probably diminished. According to Sarkaria and Hammer (7), true lactic acid bacteria grown in milk at different temperatures produced no measurable changes other than variations in total micro-population and acidity.

DISCUSSION

The results show that active cultures of *L. helveticus* and *Str. thermophilus* could be prepared with considerable latitude in incubation time and temperature. Variations within limits in methods of incubation of stock cultures can be employed with assurance that satisfactory growth and fermentation of cultures will be maintained. When large masses of cells, or bulk cultures, are required, similar variations in incubation time and temperature could be employed; and, in addition, if only a portion of the bulk culture is used, the remainder can be stored for short periods at low temperatures without loss in vitality. Thus time may be saved by the preparation of large quantities of bulk culture and punctuality in transferring may not be necessary, even though starter cultures may be needed every day or twice a day.

The results obtained in this study may not indicate the effects of these variations on the heat resistance of starter bacteria. This phase of the problem will be discussed in the following paper of this series.

SUMMARY

1. For mother cultures transferred successively at either 37°, 40°, or 42° C., the incubation time of *L. helveticus* and *Str. thermophilus* may be varied as much as 12 hours beyond an initial incubation period of 12 hours without harmful effect on culture activity. Cultures transferred every 12 or 24 hours produced similar populations and acidities when 12-hour bulk cultures made from them were compared at 37°.

2. Continuous incubation at 45° with transfers every 12 or 24 hours produced cultures of *Str. thermophilus* which were equivalent in activity to those grown at either 37°, 40°, or 42° C. Cultures of *L. helveticus* grew irregularly and poorly after successive transfers at 45°.

3. After incubation at 37° from 12 to 16 hours, or at 42° from 12 to 24 hours, cultures of *L. helveticus* could be stored at 0° to 20° C. until they were 96 hours old without reduction in their activity.

4. Cultures of *Str. thermophilus* incubated at 37° from 9 to 16 hours, or at 42° from 12 to 24 hours, could be continued at 0° or 20° C. until they

were 96 hours old without becoming less active than cultures transferred every 12 hours at 37° or 42° C.

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FACTORS AFFECTING THE ACTIVITY AND HEAT RESISTANCE OF SWISS CHEESE STARTER CULTURES. IV. EFFECT OF VARIATIONS IN TIME AND TEMPERATURE OF INCUBATION AND OF STORAGE ON HEAT RESISTANCE OF CULTURES*

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Improved methods of handling and preparing starter cultures of thermoduric lactic acid bacteria are useful to dairy manufacturers, especially to makers of Swiss cheese to whom the activity and heat resistance of the bacteria are of great importance. There is frequently a desire to modify the method of preparation of starter cultures, but the uncertainty of the effect the change may have on the vitality of the bacteria has limited attempts to vary customary procedures.

The present study was undertaken to determine to what extent time and temperature of incubation and of storage could be varied without loss in satisfactory activity and heat resistance of *Lactobacillus helveticus* and *Streptococcus thermophilus*, bacteria commonly used in dairy manufacture.

Previous investigations by us (13) have shown that incubation periods at a constant temperature near the optimum for growth, or in combination with storage at low temperatures, may be varied considerably without reduction of vitality of *L. helveticus* and *Str. thermophilus*. In these experiments the heat resistance of mother cultures was not determined. Brief studies by Elliker (4) demonstrated that cultures grown at 37° or 40° C. for 12 hours and promptly stored for the same time at 10° were equally as heat resistant as cultures transferred every 12 hours at 37° or 40° C.

Elliker and Frazier (5) observed that 12 and 16-hour cultures of *L. helveticus* developed better after a severe heat treatment than 7 or 8-hour cultures. They also established that greater resistance was exhibited by cultures incubated at 37° or 40° C. than by those grown at 30°, 35°, or 42° C. Six, 12 and 16-hour cultures of *Str. thermophilus* were equal in heat resistance when grown at 37°, but differences appeared when incubation was at 40°. Cultures grown at 30°, 35°, or 37° C. were more heat resistant than those incubated at 40° or 42° C.

Comprehensive investigations of factors affecting heat resistance of nonsporeforming bacteria appear in the literature. Such factors as temperature of incubation, age of culture, character of culture medium, and condi-

Received for publication March 10, 1941.

* The Wisconsin Alumni Research Foundation provided a grant for this work. It is published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

tions of the heat treatment have been reviewed and studied by Anderson and Meanwell (1), Brown and Peiser (2), Claydon (3), Elliker (4), Elliker and Frazier (5), Fay (7), Peppler (12), and Robertson (14).

EXPERIMENTAL

Lactobacillus helveticus, Strain 39aW, and *Streptococcus thermophilus*, Strain C-3, the organisms used in studies by Elliker and Frazier (5, 6) and by us (13), were employed in the major portion of this investigation. In part of the work the heat resistance of the same strain of *L. helveticus* and Strain Mc of *Str. thermophilus*, both grown in symbiosis with a film yeast, *Candida krusei*, commonly called a "mycoderm," were compared with unassociated cultures of these bacteria.

Except for minor modifications the methods and apparatus used by Elliker and Frazier (5) were adopted. Mother cultures were grown in freshly autoclaved reconstituted skimmilk, designated as the "normal" medium. Since the heat resistance of *L. helveticus* is influenced by the quality of the medium, as shown by Elliker and Frazier (6), the organism also was grown in a "fortified" medium composed of freshly autoclaved reconstituted skimmilk and 0.1 per cent Neopeptone. *Str. thermophilus* was carried in the fortified medium in some studies. After seven to nine consecutive culture generations, final transfers were made to a larger quantity of medium to facilitate removal of samples for heat treatment and determinations of acidity. These cultures were designated as inoculating cultures. Heat resistance of cultures was measured in terms of rapid growth and acid production of subcultures in the normal skimmilk medium following a heat treatment simulating that encountered during Swiss cheese manufacture. Subcultures of *L. helveticus* were heated at 60° or 62° C. for 30 minutes; subcultures of *Str. thermophilus* were subjected to 62° or 64° C. for the same period. In most studies the higher temperature mentioned for each organism was adopted to emphasize differences in the activity of cultures. Plate counts were substituted for the direct microscopic method used by Elliker and Frazier to determine the number of viable cells directly before and after heating, and the rate of growth following the heat treatment. Plates were poured in triplicate using the medium of Kulp and White (9). Heat-treated subcultures were incubated at 37° to avoid the long chains which these bacteria form at higher temperatures. The amount of decrease in pH of the incubated culture during a definite period after heating was used as a measure of activity of surviving cells.

Influence of 12 and 24-hour successive transfers of mother cultures on heat resistance. The temperatures selected for incubation of the mother cultures of *L. helveticus* and *Str. thermophilus* were 37° and 40° C., since it had been found that heat resistant cultures were produced at both tem-

peratures, although the higher temperature was better under some conditions. Mother cultures of each organism were transferred every 12 and 24 hours. *L. helveticus* was grown in two different media. reconstituted skimmilk, or "normal" medium, and the same medium with "Neopeptone" added. Cultures of *Str. thermophilus* were grown in the fortified medium when it was observed that cultures carried in the normal medium developed a low resistance to heat.

TABLE 1

Influence of kind of culture medium and of successive transfers[1] of mother cultures of Lactobacillus helveticus and Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures			Heat-treated subcultures			
Incubation time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Temp. of heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	°C.	
L. helveticus						
12 hrs. 37°	normal[2]	0.85	2.34	2.17	60	0.39
12 hrs. 37°	fortified[3]	0.89	2.47	2.23	60	0.48
24 hrs. 37°	normal	1.27	2.23	1.74	60	0.36
24 hrs. 37°	fortified	1.27	2.50	1.99	60	0.47
12 hrs. 40°	normal	1.13	2.66	2.46	62	0.53
12 hrs. 40°	fortified	1.09	2.08	2.02	62	0.53
24 hrs. 40°	normal	1.47	2.44	2.35	62	0.49
24 hrs. 40°	fortified	1.25	1.75	1.61	62	0.44
Str. thermophilus						
12 hrs. 37°	normal	0.71	0.614	0.407	62	1.22
24 hrs. 37°	normal	0.88	0.560	0.357	62	1.16
12 hrs. 40°	normal	0.72	0.585	0.220	62	0.98
12 hrs. 40°	fortified	0.90	0.720	0.490	62	1.26
24 hrs. 40°	normal	0.87	0.290	0.069	62	0.95
24 hrs. 40°	fortified	0.91	0.405	0.232	62	0.85

[1] Seven to nine consecutive transfers.
[2] Reconstituted skimmilk.
[3] Neopeptone-skimmilk medium.

The effect of successive transfers on the activity of heat-treated subcultures, as shown in table 1, varies not only with incubation temperature but also the quality of the medium. In order to limit the extensive data concomitant with hourly determinations of plate counts and acidity after heating, the plate counts directly before and after the heat treatment are reported, together with the amount of decrease in pH after six or seven hours. Mother cultures of *L. helveticus* grown in a fresh skimmilk medium at 40°, were more heat resistant than similar cultures developed at 37°. But in the fresh Neopeptone-skimmilk medium no significant differences in activity

after the heat treatment were exhibited by cultures grown either at 37° from 12 to 24 hours or at 40° for 12 hours. When *Str. thermophilus* was transferred every 12 and 24 hours at 37°, the cultures were equivalent to each other and more heat resistant than similar cultures carried at 40°. Growth in a fortified medium, however, provided 12-hour mother cultures at 40° which were equally as active after heating as those grown at 37°.

Influence of time and temperature of storage of mother cultures after incubation on heat resistance. Since the heat resistance of mother cultures was unaffected by certain limited variations in the incubation time during growth at a constant temperature, it was believed that such active cultures could be stored at low temperatures for limited periods without a reduction in heat resistance. *L. helveticus* and *Str. thermophilus* were incubated at 37° and 40° C. for periods of 6 to 24 hours and then promptly placed at 20°, 12°, or 4° C. for different periods of time. A constant temperature water bath was used for storage at 20°; an icebox having an average temperature of 12°, and a refrigerator with an average temperature of 4° provided the other means of storage.

The effect of storage time and temperature upon heat resistance of *L. helveticus* initially incubated at 37° for 12 or 24 hours is shown in table 2. Storage at 12° resulted in cultures with greater activity after a severe heat treatment than storage at 20° or 4° C. Storage time could be varied widely without significant reduction in activity of heat-treated subcultures. When *L. helveticus* was held at 37° for 12 hours, cultures could be stored at 12° for 60 hours and still be as heat resistant as the most active culture transferred every 12 hours at 37° C. Cultures incubated 24 hours at 37° and stored 48 hours at 12° were equally as resistant as those just described. When cultures were grown in a fresh Neopeptone-skimmilk medium before storage, they were more active after heating than similar cultures in the normal medium, but storage time was not prolonged beyond limits previously observed.

Storage of *Str. thermophilus*, after incubation at 37°, by methods similar to those just described for *L. helveticus*, resulted in heat resistant cultures at all low storage temperatures used, but, as the data in table 3 indicate, storage time at 12° could be varied more widely than at 4° or 20° C. Cultures which had been carried in a skimmilk medium with added Neopeptone showed more activity after the heat treatment than cultures which had been carried in the normal skimmilk medium. When *Str. thermophilus* was grown in the fortified medium, cultures carried at 37° for 12 or 24 hours followed by storage at 12° for 84 or 24 hours, respectively, as well as cultures grown 12 hours at 37° and continued for 36 hours at 4° C. were equivalent in activity after heating to cultures transferred serially at 37° in the normal medium.

According to data of table 4, *L. helveticus* incubated at 40° from 8 to 15 hours could be held for two days at low temperatures without decrease in heat resistance. When mother cultures were carried in the normal medium, incubation periods of 10 to 15 hours at 40° could be followed by 38 and 33 hours, respectively, at 20° or 12° C. In the medium with added Neopeptone, incubation periods at 40° for as little as 8 hours did not cause a reduction in heat resistance. The most active cultures in these experiments were equivalent in heat resistance to cultures transferred serially at either 37° or 40° C.

Previous investigations demonstrated that cultures of *Str. thermophilus* grown at 40° were less active after heat treatment than those carried at 37°. It could be expected that cultures stored at low temperatures after incubation at 40° would be no better than cultures transferred continuously at

TABLE 2

Influence of kind of culture medium, incubation time at 37° C., and storage time and temperature of mother cultures of Lactobacillus helveticus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures				Heat-treated subcultures			
Incubation time at 37°	Storage time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Temp. of heating	Drop in pH seven hours after heating
hours			per cent	millions/ml.	millions/ml.	°C.	
12	none	normal	0.85	2.34	2.17	60	0.39
	none	fortified	0.89	2.47	2.23	60	0.48
	36 hrs. 20°	normal	1.04	1.14	0.85	60	0.36
	36 hrs. 20°	fortified	1.07	1.45	1.21	60	0.39
	36 hrs. 12°	normal	0.97	2.48	2.55	62	0.50
	36 hrs. 12°	fortified	0.98	2.69	2.52	62	0.53
	12 hrs. 4°	normal	0.96	3.15	2.65	62	0.38
	36 hrs. 4°	normal	0.92	2.99	2.41	62	0.39
	84 hrs. 20°	normal	1.20	2.02	1.96	60	0.22
	84 hrs. 20°	fortified	1.30	2.17	2.04	60	0.28
	60 hrs. 12°	normal	1.22	2.02	2.16	62	0.46
	84 hrs. 12°	normal	1.06	1.89	1.69	62	0.37
	60 hrs. 4°	normal	0.97	2.62	2.45	62	0.43
	156 hrs. 4°	normal	0.91	2.93	2.57	62	0.31
24	none	normal	1.27	2.23	1.74	60	0.36
	none	fortified	1.27	2.50	1.99	60	0.47
	24 hrs. 20°	normal	.....	2.90	2.56	60	0.36
	24 hrs. 20°	fortified	1.45	2.86	2.73	60	0.39
	24 hrs. 12°	normal	1.29	2.12	2.16	62	0.46
	24 hrs. 12°	fortified	1.21	2.04	1.79	62	0.41
	48 hrs. 12°	normal	1.46	2.26	2.21	62	0.46
	48 hrs. 4°	normal	1.41	3.20	2.74	62	0.43
	72 hrs. 20°	normal	1.35	1.81	1.28	60	0.22
	72 hrs. 20°	fortified	1.44	1.67	1.61	60	0.30
	72 hrs. 12°	normal	1.42	3.15	2.63	62	0.42
	144 hrs. 4°	normal	1.25	2.82	2.52	62	0.26

40°. This presumption was substantiated by the results presented in table 5. Only cultures carried in a medium better than fresh reconstituted skimmilk were as active after heating as the most heat resistant cultures developed at 37°. Cultures incubated in the fortified medium at 40° from 6 to

TABLE 3

Influence of kind of culture medium, incubation time at 37° C., and storage time and temperature of mother cultures of Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures				Heat-treated subcultures			
Incubation time at 37°	Storage time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Temp. of heating	Drop in pH six hours after heating
hours			per cent	millions/ml.	millions/ml.	°C.	
12	none	normal	0.72	0.614	0.407	62	1.05
	36 hrs. 20°	normal	0.82	0.640	0.482	62	1.04
	84 hrs. 20°	normal	1.05	0.750	0.378	62	1.12
	156 hrs. 20°	normal	1.01	0.414	0.309	62	0.85
	12 hrs. 12°	normal	0.74	0.727	0.060	64	0.85
	12 hrs. 12°	fortified	0.88	0.809	0.395	64	1.04
	36 hrs. 12°	normal	0.76	0.600	0.155	64	0.88
	36 hrs. 12°	fortified	0.91	0.635	0.133	64	1.13
	84 hrs. 12°	normal	0.84	0.550	0.025	64	0.99
	84 hrs. 12°	fortified	0.94	0.485	0.074	64	1.09
	156 hrs. 12°	normal	0.83	0.675	0.155	64	0.78
	156 hrs. 12°	fortified	0.96	0.780	0.174	64	0.99
	12 hrs. 4°	normal	0.73	0.770	0.043	64	0.53
	12 hrs. 4°	fortified	0.89	0.985	0.188	64	1.02
	36 hrs. 4°	normal	0.68	0.680	0.080	64	0.91
	36 hrs. 4°	fortified	0.82	0.770	0.229	64	1.13
	60 hrs. 4°	normal	0.74	0.612	0.103	64	0.50
10	14 hrs. 12°	normal	0.70	0.724	0.110	64	0.85
	14 hrs. 12°	fortified	0.82	0.867	0.380	64	1.11
24	none	normal	0.84	0.560	0.357	62	1.00
	24 hrs. 20°	normal	0.86	0.518	0.352	62	0.96
	72 hrs. 20°	normal	1.05	0.621	0.251	62	0.97
	24 hrs. 12°	normal	0.83	0.585	0.070	64	0.74
	24 hrs. 12°	fortified	0.95	0.590	0.250	64	1.05
	72 hrs. 12°	fortified	1.02	0.940	0.337	64	0.57
	48 hrs. 4°	normal	0.89	0.560	0.235	64	0.09

7 hours, and stored at either 12° or 4° C. from 42 to 41 hours, respectively, exhibited a remarkably high heat resistance. The increased resistance of *L. helveticus* and *St. thermophilus* after growth in fortified skimmilk media may, in a few instances, be due to greater numbers of cells surviving the heat treatment. In most instances marked differences in plate counts of survivors were not observed, regardless of the method of comparison used. Whether increases in heat resistance were the result of a greater accumula-

tion of mature, heat resistant cells in a favorable medium than in a poor medium, as Elliker and Frazier (5) suggest, or whether the growth stimulants merely improve the general healthiness of the cells was not revealed by this investigation.

TABLE 4

Influence of kind of culture medium, incubation time at 40° C., and storage time and temperature of mother cultures of Lactobacillus helveticus on the activity of subcultures at 37° C. following heat treatment at 62° C. for 30 minutes

Inoculating cultures				Heat-treated subcultures		
Incubation time at 40°	Storage time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
hours			per cent	millions/ml.	millions/ml.	
10	14 hrs. 20°	normal	1.05	2.42	2.15	0.49
10	38 hrs. 20°	normal	1.21	2.66	2.50	0.46
15	9 hrs. 20°	normal	1.27	2.02	2.50	0.46
15	33 hrs. 20°	normal	1.34	3.10	2.56	0.46
10	14 hrs. 12°	normal	0.87	2.76	2.18	0.48
10	14 hrs. 12°	fortified[1]	1.13	2.78	2.67	0.59
12	12 hrs. 12°	normal	1.03	2.37	2.30	0.51
12	12 hrs. 12°	fortified[1]	1.13	2.39	2.48	0.48
8	40 hrs. 12°	normal	0.86	2.36	1.83	0.36
8	40 hrs. 12°	fortified[1]	1.03	2.71	2.49	0.51
10	38 hrs. 12°	normal	1.00	2.42	2.21	0.52
15	33 hrs. 12°	normal	1.29	2.28	2.26	0.50
8	64 hrs. 12°	normal	0.95	2.45	2.12	0.42
15	57 hrs. 12°	normal	1.29	2.94	2.39	0.39
10	14 hrs. 4°	normal	0.92	2.26	2.28	0.37
15	9 hrs. 4°	normal	1.25	1.96	2.44	0.48
9	39 hrs. 4°	normal	0.87	2.63	2.09	0.44
9	39 hrs. 4°	fortified[1]	1.00	2.57	2.23	0.53
10	38 hrs. 4°	normal	0.92	2.39	2.25	0.37
15	33 hrs. 4°	normal	1.22	2.58	2.99	0.37
12	none	normal	1.13	2.66	2.46	0.39
12	none	fortified[2]	1.09	2.08	2.02	0.48
24	none	normal	1.47	2.44	2.35	0.36
24	none	fortified[2]	1.25	1.75	1.61	0.47

[1] Malt extract—skimmilk medium.
[2] Neopeptone—skimmilk medium.

Influence of a film yeast ("mycoderm") on heat resistance of bacteria grown in symbiosis with it. Several cultures in which the thermoduric lactic acid bacteria are associated with a film yeast, *Candida krusei*, sometimes called a "mycoderm," are used commonly by makers of Swiss cheese. In such cultures the "mycoderm" is known to increase viability of bacteria by reduction of the acidity (8, 11), and with some species of bacteria the production of lactic acid is stimulated (8, 10). Reports concerning the effect of a film yeast on thermal resistance of these bacteria have not been found. Comparisons of heat resistance were made between pure cultures of bacteria and the same cultures associated with a film yeast. *L. helveticus*, Strains 39aW and 39aW-my, and *Str. thermophilus*, Strains Mc and Mc-my,

TABLE 5

Influence of kind of culture medium, incubation time at 40° C., and storage time and temperature of mother cultures of Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment at 64° C. for 30 minutes

Inoculating cultures				Heat-treated subcultures		
Incubation time at 40°	Storage time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Drop in pH six hours after heating
hours			per cent	millions/ml.	millions/ml.	
8	16 hrs. 20°	normal	0.75	0.655	0.025	0.27
12	12 hrs. 20°	normal	0.79	0.520	0.025	0.27
15	9 hrs. 20°	normal	0.83	0.535	0.015	0.29
8	40 hrs. 20°	normal	0.81	0.570	0.065	0.52
15	33 hrs. 20°	normal	0.88	0.588	0.150	0.57
8	40 hrs. 12°	normal	0.72	0.589	0.153	0.66
15	33 hrs. 12°	normal	0.80	0.545	0.082	0.46
6	42 hrs. 12°	normal	0.66	0.653	0.064	0.53
8	64 hrs. 12°	normal	0.77	0.595	0.111	0.37
15	57 hrs. 12°	normal	0.84	0.555	0.100	0.37
8	40 hrs. 4°	normal	0.68	0.587	0.253	0.84
15	33 hrs. 4°	normal	0.79	0.661	0.031	0.28
7	41 hrs. 4°	normal	0.58	0.530	0.030	0.48
8	16 hrs. 4°	normal	0.71	0.520	0.060	0.60
15	9 hrs. 4°	normal	0.82	0.428	0.044	0.41
8	64 hrs. 4°	normal	0.65	0.730	0.044	0.52
12	60 hrs. 4°	normal	0.73	0.580	0.042	0.56
6	42 hrs. 12°	fortified[1]	0.82	0.645	0.333	1.25
7	41 hrs. 4°	fortified[1]	0.78	0.780	0.268	1.14
12	none	fortified[1]	0.90	0.720	0.490	1.26

[1] Neopeptone—skimmilk medium.

were selected. Strains 39aW-my and Mc-my were associated with *Candida krusei*. Methods of handling mother cultures described above were employed. Before the removal of samples for transfer to fresh media or for heat treatment, cultures were shaken to obtain a uniform mixture of film yeast and bacteria. The "mycoderm" was destroyed during the heat treatment; consequently, it did not interfere with plate counts or subsequent fermentation in the subcultures.

Results with *L. helveticus*, table 6, demonstrate that incubation for short consecutive periods at 37° C., and similar incubation periods followed by storage at 20° C. for 36 hours produced no differences in heat resistance between symbiotic and pure cultures. But when starters were incubated for 72 to 96 hours at 25° or 12-hour (37°) cultures were stored for 154 hours at 20°, the associated cultures were significantly more heat resistant than the pure cultures. It was observed that bacteria nearest the surface growth of the film yeast were far more active after heat treatment than bacteria farthest removed from the top of the mixed culture.

Similar treatment of *Str. thermophilus* also revealed that associated cul-

TABLE 6

Influence of incubation time and temperature of mother cultures of Lactobacillus helveticus (39aW) grown with a "mycoderm" (my) on the activity of subcultures at 37° C. following heat treatment at 62° C. for 30 minutes

Inoculating cultures			Heat-treated subcultures		
Type of mother culture	Incubation time and temperature	Titratable acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	
39aW	12 hrs. 37°	0.89	2.67	2.15	0.30
39aW-my	12 hrs. 37°	0.92	1.64	1.17	0.30
39aW	12 hrs. 37° 36 hrs. 20°	0.98	1.93	1.97	0.43
39aW-my	12 hrs. 37° 36 hrs. 20°	1.17	2.07	1.98	0.43
39aW	72 hrs. 25°	1.20	1.58	1.71	0.37
39aW-my	72 hrs. 25°	1.24	1.20	1.76	0.44
39aW	96 hrs. 25°	1.42	1.90	1.80	0.45
39aW-my	96 hrs. 25°	1.37	2.11	1.64	0.53
39aW	14 hrs. 37° 154 hrs. 20°	1.27	2.80	2.80	0.41
39aW-my	14 hrs. 37° 154 hrs. 20°				
*(a)		1.18	2.18	2.36	0.68
(b)		1.56	2.01	2.06	0.55
(c)		1.28	2.12	1.89	0.62

* (a) Subculture from topmost quarter of inoculating culture.
(b) Subculture from bottom quarter of inoculating culture.
(c) Subculture from inoculating culture previously thoroughly shaken to mix growth of top and bottom areas.

tures possessed greater activity after heating than pure cultures of this streptococcus. As shown in table 7 heat resistant mixed cultures were obtained when they were carried at 37° for 12 hours or at 25° for 72 hours. The influence of the "mycoderm" was not overcome by the number of successive transfers at 37° used here. In some instances increased activity may be attributable to higher numbers of survivors. It is also evident from data of table 7 that bacteria in the vicinity of "mycoderm" growth were more active after heat treatment than those farthest from the surface film.

The role played by *Candida krusei* resulting in enhanced heat resistance of these lactic starter bacteria will be discussed in another paper.

A summary of variations in time and temperature of incubation and storage which provided starter cultures of equivalent and maximum heat resistance appears in table 8.

DISCUSSION

The data support the belief that a variety of modifications can be applied to methods for handling starter cultures of thermoduric bacteria, depending

TABLE 7

Influence of incubation time and temperature of mother cultures of Streptococcus thermophilus (Mc) grown with a "mycoderm" (my) on the activity of subcultures at 37° C. following heat treatment at 64° C. for 30 minutes

Inoculating cultures			Heat-treated subcultures		
Type of mother culture	Incubation time and temperature	Titratable acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	
Mc	12 hrs. 37°	0.62	0.560	0.075	0.42
Mc-my	12 hrs. 37°	0.64	0.565	0.175	0.60
Mc	72 hrs. 25°	0.71	0.435	0.205	1.17
Mc-my	72 hrs. 25°	0.69	0.545	0.390	1.25
Mc	96 hrs. 25°	0.68	0.484	0.096	1.04
Mc-my	96 hrs. 25°	0.64	0.449	0.098	1.04
Mc	12 hrs. 37° 36 hrs. 20°	0.61	0.448	0.010	0.45
Mc-my	12 hrs. 37° 36 hrs. 20°	0.64	0.599	0.020	0.48
Mc	14 hrs. 37° 154 hrs. 20°	0.83	0.628	0.318	0.30
Mc-my	14 hrs. 37° 154 hrs. 20°				
*(a)		0.75	0.425	0.090	0.89
(b)		0.91	0.505	0.085	0.11
(c)		0.73	0.415	0.105	0.41

* (a) Subculture from topmost quarter of inoculating culture.
(b) Subculture from bottom quarter of inoculating culture.
(c) Subculture from inoculating culture previously thoroughly shaken to mix growth of top and bottom areas.

upon incubation temperature used, storage temperature available, and quality of the culture medium employed, without lessening the activity and heat resistance of the bacteria.

L. helveticus and *Str. thermophilus* may be grown in a good medium such as reconstituted skimmilk at a common temperature, 37°, to develop cultures of high heat resistance. If a better culture medium, one with added accessory substances is used, the heat resistance of cultures is not only assured but actually increased. This modification of methods for handling starter may be adopted when higher than usual cooking temperatures of curd are employed to avoid undesirable gas formation in Swiss cheese. The results of this investigation indicate that under such circumstances starter of great heat resistance can be produced by improving the culture medium, by incubation at 40° instead of 37° C., or by combining both procedures. In the case of *Str. thermophilus,* however, growth at 40° requires a medium of highest quality, and more careful control of incubation time is necessary to provide cultures of proper maturity. The results again emphasize the importance of a suitable and uniform starter culture medium, suggesting adoption of a definite plan of selection of high quality milk for this purpose.

TABLE 8

A summary of variations in time and temperature of incubation and storage which provided cultures of equivalent and maximum heat resistance

Starter	Incubation time and temperature	Quality of skim-milk medium
L. helveticus strain 39aW	12 hrs. 37° + 60 hrs. 12° 24 hrs. 37° + 48 hrs. 12° 10–15 hrs. 40° + 38 hrs. 20° or 12°	normal*
	12–24 hrs. 37° or 40° 12 hrs. 37° + 36 hrs. 12° 8– 9 hrs. 40° + 40 hrs. 12° or 4° 10–12 hrs. 40° + 12 hrs. 12° 15 hrs. 40° + 9 hrs. 4°	fortified**
Str. thermophilus strain C-3	12–24 hrs. 37° 12 hrs. 37° + 84 hrs. 20° 12 hrs. 37° + 36 hrs. 12°	normal*
	12 hrs. 37° or 40° 10–12 hrs. 37° + 84 hrs. 12° 12 hrs. 37° + 36 hrs. 4° 24 hrs. 37° + 24 hrs. 12° 6– 7 hrs. 40° + 42 hrs. 12° or 4°	fortified**

* Mother cultures grown in reconstituted skimmilk medium.
** Cultures carried in skimmilk medium with accessory substances added.

Results obtained with cultures transferred every 24 hours at either 37° or 40° C. suggest that starter cultures of *L. helveticus* and *Str. thermophilus* need not be transferred twice daily, as it is done in many cheese factories, to obtain heat resistant bacteria. The preparation of bulk starter for Swiss cheese need not be a daily task. With adequate incubation and storage facilities, bulk starter grown at 37° for 12 or 24 hours, for example, can be held from one to two days in a factory cold room. Thus enough starter can be prepared to supply the need for two days' manufacture. When cheese is made twice daily, starter for both lots can be prepared at the same time, a portion to be used in the morning and the remainder held in the cold room for use in cheese made in the afternoon.

The increased heat resistance of *L. helveticus* and *Str. thermophilus* grown in association with the film yeast, *Candida krusei*, revealed an aspect of symbiosis not recognized previously. Limited studies suggest the possibility that heat resistant cultures of these bacteria may be developed at temperatures considerably below the optimum for thermoduric bacteria, such as 30° C. It was demonstrated that bacteria near the "mycoderm" pellicle were more active after heating than bacteria from the same culture but at the lowest level, and that the beneficial influence of the film yeast extended for some distance below the surface of the culture. These results suggest that heat resistant cultures of these bacteria may be obtained by methods other than those customarily employed; such as, growing asso-

ciated cultures in thin layers of media, or in media with increased surface area.

Although the data have been discussed only in regard to their use by workers in the Swiss cheese industry, others who prepare cultures of thermoduric lactic acid bacteria required to grow rapidly after severe heat treatments may find helpful modifications of methods for handling such bacteria.

SUMMARY

1. Successive transfers every 12 and 24 hours at 37° and 40° C. resulted in more heat resistant cultures of *L. helveticus* at 40° than at 37° when mother cultures were grown in freshly reconstituted skimmilk. In a medium with added accessory substances, such as Neopeptone or malt extract, 12 to 24 hours at 37° and 12 hours at 40° gave best results.

2. Consecutive transfers of *Str. thermophilus* at a constant temperature led to most active cultures when incubation in the fortified medium varied from 12 to 24 hours at 37°; but at 40° highest resistance to heat was shown by 12-hour cultures carried in the fortified medium.

3. Storage at 12° was least harmful to *L. helveticus* following incubation at 37°. Greatest heat resistance was observed when 12-hour cultures at 37° were held at 12° no longer than 60 hours, and 24-hour cultures at 37° could be stored for 48 hours at 12°. Improvement of the skimmilk medium with Neopeptone increased slightly the heat resistance of stored mother cultures.

4. *Str. thermophilus* grown in the fortified medium at 37° for 10 to 12 hours and then stored 84 hours at 12° or 36 hours at 4°, and 24-hour cultures at 37° held 24 hours at 12° were equivalent in heat resistance to cultures transferred every 12 hours at 37°. Storage of cultures at 12° was better than at either 4° or 20° C.

5. After incubation at 40° for 8 to 15 hours, *L. helveticus* could be stored at 20°, 12°, or 4° C. until cultures were 48 hours old. Storage at 12° appeared to be least harmful to mother cultures.

6. When grown at 40° C. *Str. thermophilus* gave heat resistant cultures only when Neopeptone was added to the skimmilk medium. Incubation at 40° for 6 to 7 hours followed by storage at either 12° or 4° C. until cultures were 48 hours old resulted in cultures as active after heating as those transferred serially at 37°.

7. Mixed cultures of the film yeast, *Candida krusei*, and *L. helveticus* exhibited greater heat resistance than pure cultures of this bacterium after incubation at 25° C. for 72 to 96 hours, and also when incubation at 37° C. for 12 hours was followed by storage at 20° C. for 36 hours. Frequent transfer of associated cultures at 37° reduced the influence of the "mycoderm"; these cultures were equal in heat resistance to pure cultures of the bacteria carried under similar conditions.

8. *Str. thermophilus*, Mc, grown with "mycoderm" was more active after heating than pure cultures of the bacteria carried under similar conditions. Numerous successive 12-hour transfers of the associated culture at 37° did not decrease the influence of the film yeast.

9. In old cultures bacteria nearest the "mycoderm" film grew and fermented better following heat treatment than bacteria in areas farthest removed from the pellicle formed by the film yeast.

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THE LIMIT OF ERROR OF THE SIMPLIFIED VACUUM SOLIDS TEST AS APPLIED TO ICE CREAM MIX, EVAPORATED AND SWEETENED CONDENSED MILK

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INTRODUCTION

A Simplified Vacuum Solids Test for determining total solids in ice cream mix, condensed and evaporated milk has been developed by the Department of Dairy Manufactures of Texas Technological College. This method was devised in order to provide the dairy industry with a method for the estimation of total solids, which is accurate enough to meet the requirements of the commercial operator, rapid enough to be of commercial value, cheap enough to be available to all who wish to perform analysis for total solids, and simple enough to be performed by the average laboratory employee.

The purpose of this work was to determine the most satisfactory procedure for operating the Simplified Vacuum Solids Test; also to determine its accuracy as compared to the Official Method for determining total solids in evaporated milk, sweetened condensed milk, and to the adapted Official Method for ice cream mix.

The Simplified Vacuum Solids Test was originally designed in 1933 by Professor K. M. Renner, Head of the Department of Dairy Manufactures, Texas Technological College. Dr. William L. Ray, formerly of the Chemistry Department of Texas Technological College, offered valuable suggestions and advice on the construction of the original apparatus.

The first application of the test was made in the analysis for total solids in egg yolk. Numerous changes from the original design and procedure have been made in order to make the test more adaptable to liquid dairy products.

A number of commercial plants have adopted the Simplified Vacuum Solids Test. Until the present work was performed, there were no data available showing a statistical comparison of the Simplified Vacuum Solids Test to the Official Method.

REVIEW OF LITERATURE

The accuracy of several methods commonly used for testing condensed milk, evaporated milk and ice cream mix for total solids has been studied by previous investigators. Among the methods commonly used are the Mojonnier, Refractometric, and various adaptations of the Official.

Received for publication March 10, 1941.

* A portion of a thesis submitted to the Graduate Faculty, Texas Technological College, in partial fulfillment for the Master of Science Degree.

In studying the accuracy of methods for testing sweetened condensed milk, Fisher and Rice (2) compared results obtained by the Official method to the Mojonnier and a Modified Official method which they devised. These workers tested twenty samples of sweetened condensed milk by all three methods, and found that in all but one instance the Official method yielded the lowest results. The Mojonnier method gave the highest results on two-thirds of the tests. On these twenty samples the Mojonnier results averaged 0.57 per cent higher than the Official, whereas the Modified method averaged only 0.3022 per cent higher.

Fisher and Watts (3) made a study of some of the methods used in testing ice cream for total solids. They compared the adapted Official method to the Modified method recommended by Fisher and Rice (2) and to the Mojonnier method. They tested twelve samples of ice cream by each of the methods listed above. On all but two samples the adapted Official method yielded the lowest results, and the Mojonnier method the highest. The Mojonnier results averaged 0.44 per cent higher than the adapted Official, and the Modified method results averaged 0.23 per cent higher. These observations substantiated the results obtained by Fisher and Rice (2) in their work on sweetened condensed milk.

Rice and Miscall (6) in studying the Refractometric method of determining total solids in sweetened condensed milk, found that if accurate results are to be obtained the determinations must be made within a few hours after the batch is drawn because of the crystallizing tendency of lactose. These workers devised a formula by which total solids could be calculated from the refractive index, but they pointed out limitations of the reliability of the method in that the casein and albumen cannot be completely precipitated.

Menefee and Overman (4) also studied the Refractometric method of testing condensed and evaporated milk for total solids, using a copper sulphate solution to precipitate the protein. These investigators developed a formula for computing the total solids from the Refractometer reading of these products. They tested sixteen samples of condensed milk and found a standard error of estimate of 0.4641, with the greatest error on any sample 0.78. Twenty-five samples of evaporated milk were tested, and the results showed a standard error of estimate of 0.49, with the largest single error being 0.97.

LABORATORY EQUIPMENT

The following laboratory equipment is required for assembling the Simplified Vacuum Solids Testing Device:

(1) 1—1000-ml. Pyrex Beaker
(2) 1—1000-ml. Suction Flask
(3) Pyrex Test Tubes 7″ long and 1″ in diameter

(4) 1—Glass Tee
(5) 1—Glass L
(6) 5—Ft. of 7-mm. Glass Tubing
(7) 1—2-oz. Jar and Mercury for Mercury Well
(8) 1—Aspirator
(9) 1—No. 8 Two-hole Rubber Stopper
(10) 1—No. 10 Two-hole Rubber Stopper
(11) 1—Bunsen Burner
(12) 3—Ft. of Heavy Rubber Tubing of suitable size to fit Glass Tee, Suction Flask and Aspirator

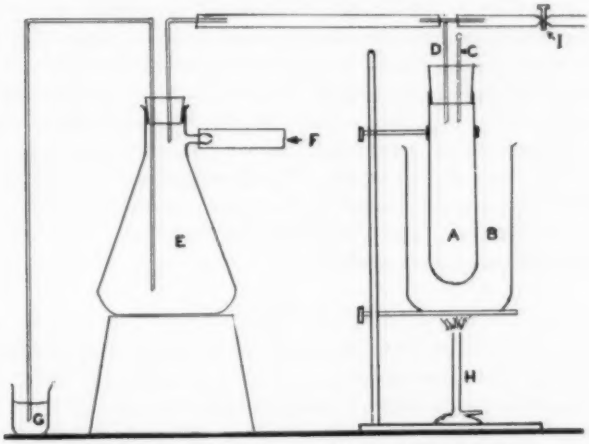

FIG. 1. Simplified vacuum solids testing apparatus.
A—1″ × 7″ pyrex test tube for sample.
B—1000-ml. beaker, water bath.
C—Thistle tube.
D—Glass T.
E—500-ml. suction flask.
F—Suction tubing to aspirator (suction pump).
G—Mercury well.
H—Bunsen burner—Heat source.
I—Pinch cock.

The materials used and the method of assembling the apparatus are shown in the accompanying illustration. The assembled system must be free from air leaks, and the water pressure sufficient to maintain a 24-inch vacuum as measured on the mercury column.

MATERIAL TESTED

All of the ice cream mix and sweetened condensed milk samples tested in this work were obtained from the College Creamery. Commercial samples of evaporated milk were purchased from local retail stores. Prior to weigh-

ing and testing, the samples were kept at a temperature of about 40° F. to minimize bacterial action and acid development. No sample was held longer than two days, that being the length of time required to perform the series of fifty tests which were performed on each sample.

PREPARATION OF SAMPLES

None of the sweetened condensed milk tested in this work had been homogenized, whereas all of the ice cream mix and evaporated milk samples had been homogenized. The sweetened condensed skim milk samples were prepared according to the recommendations of the Official method (1) for total solids determination. An attempt was made to prepare the sweetened condensed whole milk samples in the same manner, but was unsuccessful because the butterfat churned when the warm diluted sample was agitated. The sweetened condensed whole milk samples were maintained at a temperature of about 50° F. while being diluted with an equal weight of cold distilled water, stirred and mixed. The homogenized ice cream mix and evaporated milk samples required no preliminary treatment other than mixing. All samples were thoroughly mixed by stirring and pouring several times before weighing each sample.

TESTING PROCEDURE

A large (1″ × 7″) test tube is placed on the scale pan of an analytical balance, and is held in an upright position by a wire clasp designed for the purpose. One to two grams of the sample is weighed into the test tube. The test tube containing the sample is then placed in the 1000-ml. beaker which contains boiling distilled water. The test tube and beaker are supported by a ring stand as shown in the illustration. The two-hole rubber stopper, into which is inserted the thistle tube and suction line, is seated in the test tube, and the water then turned into the aspirator.

As the vacuum is created in the test tube, the sample boils vigorously and spreads in a thin film over the interior of the test tube. Care must be exercised at this point to prevent any of the sample from escaping into the suction line. This is controlled by using proper volume and dilution of sample, and if necessary temporarily breaking the vacuum. The sample is thus heated for fifteen minutes, then removed from the boiling water, dried thoroughly, cooled in a desiccator and weighed. The per cent total solids is calculated by dividing the weight of residue by the weight of sample and multiplying by 100. A proper correction must be made according to the amount of the original dilution.

EXPERIMENTAL

A large number of preliminary analyses was performed in an attempt to improve on the procedure previously recommended by Renner (5) in

which the weighings were performed on a Torsion balance. In the operation of the Simplified Vacuum Solids Test, the best results were obtained when a sample weighing from one to two grams was used, diluted in such a manner as to contain between 18 per cent and 35 per cent total solids, heated at 212° F. under at least a 24-inch vacuum for fifteen minutes, and cooled in a desiccator before weighing.

In order to determine the accuracy of the Simplified Vacuum Solids Test, groups of fifty tests for total solids were performed on each of the following products: Evaporated Milk, Ice Cream Mix, Sweetened Condensed Whole Milk and Sweetened Condensed Skim Milk.

Total solids determinations were also made by the Official method (1) or in the case of ice cream mix, an adapted Official method (2). The error of the Simplified Vacuum Solids method was measured from the average of four Official results on each sample.

For the purpose of measuring the accuracy of the results, the following statistical methods were applied to all groups of tests:

1. The arithmetic mean of error was determined on the error and on the total solids.
2. The standard deviation was determined on the error and on the total solids.
3. The coefficient of variation was determined on the total solids.

The accuracy of the Simplified Vacuum Solids Test as compared to the Official method for determining the total solids in evaporated milk, ice cream mix, sweetened condensed whole milk and sweetened condensed skim milk is shown in tables 1, 2, 3 and 4, respectively.

DISCUSSION AND CONCLUSIONS

One of the greatest difficulties in the operation of the Simplified Vacuum Solids Test is the prevention of the loss of small particles of sample into the suction line. This difficulty can be controlled by using a small sample (one or two grams) which has been diluted so as to contain between 18 per cent and 35 per cent total solids. Low solids samples have more tendency to be drawn into the suction line, whereas high solids samples having a high viscosity do not spread in a thin uniform film over the interior of the surface of the test tube, and thus have less evaporation surface.

The arithmetic mean of the error and of the total solids was determined in order to show the simple average of the error. The standard deviation was used in comparing the error of the results, because it is a measure of absolute variability. The coefficient of variability was also calculated, since it shows the ratio of the standard deviation to the arithmetic mean.

In estimating the total solids in evaporated milk, the Simplified Vacuum Solids method compared favorably with other methods in use, in that the arithmetic mean of error was 0.1529.

TABLE 1

Evaporated milk

Official T. S. = 26.57%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	2.0539	.5487	26.72	.15
2	2.0137	.5369	26.66	.09
3	2.0145	.5380	26.71	.14
4	1.9976	.5321	26.64	.07
5	2.0113	.5356	26.62	.05
6	2.0065	.5340	26.61	.04
7	1.9418	.5200	26.77	.20
8	1.9404	.5190	26.74	.17
9	1.8876	.5035	26.67	.10
10	1.8689	.4989	26.69	.12
11	1.8749	.5017	26.75	.18
12	1.8705	.5005	26.75	.18
13	1.8896	.5058	26.74	.17
14	1.9391	.5193	26.78	.21
15	2.0827	.5570	26.74	.17
16	2.1160	.5654	26.72	.15
17	2.0820	.5548	26.64	.07
18	2.0396	.5462	26.78	.21
19	2.0342	.5443	26.76	.19
20	2.0576	.5506	26.76	.19
21	1.9757	.5277	26.71	.14
22	2.0303	.5412	26.66	.09
23	1.9553	.5251	26.85	.28
24	2.0012	.5347	26.72	.15
25	1.9779	.5290	26.75	.18
26	1.9701	.5278	26.78	.21
27	1.9333	.5176	26.77	.20
28	1.9750	.5294	26.78	.21
29	1.9638	.5259	26.78	.21
30	2.1213	.5647	26.62	.05
31	2.0613	.5500	26.68	.11
32	2.0344	.5427	26.68	.11
33	2.0046	.5333	26.60	.03
34	2.0279	.5412	26.69	.12
35	1.9319	.5172	26.77	.20
36	1.9926	.5334	26.77	.20
37	1.9467	.5208	26.75	.18
38	1.9563	.5237	26.76	.19
39	1.9365	.5090	26.75	.18
40	1.8891	.5058	26.77	.20
41	1.9449	.5202	26.75	.18
42	1.9555	.5231	26.75	.18
43	1.9386	.5181	26.73	.16
44	1.9120	.5128	26.82	.25
45	1.9318	.5171	26.76	.19
46	1.8735	.5030	26.84	.27
47	2.1404	.5690	26.58	.01
48	2.0434	.5456	26.70	.13
49	2.0250	.5386	26.60	.03
50	2.1125	.5655	26.72	.15

Statistical analysis of results in table 1

1. The arithmetic mean of error = 0.1529.
2. The standard deviation of the error = 0.0624.
3. The arithmetic mean of the total solids = 26.72.
4. The standard deviation of the total solids = 0.0624.
5. The coefficient of variation of the total solids = 0.2335.
6. No minus errors were obtained.
7. The range of error was from 0.01 to 0.28.
8. Ninety-six per cent of the results were within 0.25 per cent of the Official.

TABLE 2

Ice cream mix

Official T. S. = 36.87%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.1645	.4360	37.44	.57
2	1.0222	.3801	37.18	.31
3	1.0284	.4041	37.33	.46
4	1.0298	.3908	37.94	1.07
5	1.0496	.3900	37.16	.29
6	1.0554	.3936	37.29	.42
7	1.1056	.4110	37.18	.31
8	1.0408	.3863	37.12	.25
9	1.0026	.3732	37.22	.35
10	1.0212	.3818	37.39	.52
11	1.0438	.3894	37.31	.44
12	1.0196	.3823	37.49	.62
13	1.0076	.4764	37.36	.49
14	1.0107	.3787	37.47	.60
15	1.0130	.3755	37.07	.20
16	1.0032	.3779	37.67	.80
17	.9979	.3740	37.48	.61
18	.9899	.3657	36.94	.07
19	1.0109	.3759	37.14	.27
20	1.0651	.3965	37.29	.42
21	1.0214	.3819	37.39	.52
22	1.0567	.3950	37.38	.51
23	1.0170	.3799	37.35	.48
24	1.0442	.3889	37.24	.37
25	1.0653	.3993	37.48	.61
26	1.0620	.3936	37.07	.20
27	.9936	.3733	37.57	.70
28	1.0250	.3855	37.71	.84
29	1.0198	.3823	37.48	.61
30	.9857	.3681	37.45	.58
31	.9973	.3725	37.36	.49
32	1.0210	.3815	37.36	.49
33	1.0301	.3887	37.73	.86
34	1.0252	.3830	37.41	.54
35	1.0221	.3804	37.21	.34
36	1.0132	.3811	37.61	.74
37	1.0060	.3788	37.65	.78
38	.9908	.3695	37.29	.42
39	.9901	.3719	37.56	.69
40	.9717	.3649	37.55	.68
41	1.0038	.3742	37.28	.41
42	.9723	.3639	37.43	.56
43	.9643	.3590	37.23	.36
44	1.0105	.3790	37.49	.62
45	.9943	.3675	36.96	.09
46	.9673	.3607	37.29	.42
47	.9504	.3589	37.76	.89
48	.9994	.3755	37.57	.70
49	1.0026	.3762	37.52	.65
50	.9647	.3580	37.11	.24

Statistical analysis of results in table 2

1. The arithmetic mean of the error = 0.5090.
2. The standard deviation of the error = 0.1521.
3. The arithmetic mean of the total solids = 37.38.
4. The standard deviation of the total solids = 0.2261.
5. The coefficient of variation of the total solids = 0.6032.
6. No minus errors occurred in the results.
7. The range of error was from 0.07 to 1.07.
8. Twelve per cent of the results were within 0.25 per cent of the adapted Official.
9. Fifty per cent of the results were within 0.50 per cent of the adapted Official.
10. Ninety-eight per cent of the results were within 1 per cent of the adapted Official.
11. Only one sample had an error in excess of 1 per cent.

TABLE 3

Sweetened condensed whole milk

Official T. S. = 70.86%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.1106	.3961	71.34	.48
2	1.1004	.3918	71.22	.36
3	1.0915	.3905	71.56	.70
4	1.1130	.3966	71.26	.40
5	1.0959	.3906	71.28	.42
6	1.0982	.3909	71.18	.32
7	1.0640	.3795	71.34	.48
8	1.0743	.3815	71.02	.16
9	1.0614	.3772	71.08	.22
10	1.0601	.3780	71.32	.46
11	1.0776	.3834	71.16	.30
12	1.0439	.3721	71.30	.44
13	1.0458	.3732	71.36	.50
14	1.1027	.3942	71.48	.62
15	1.0841	.3875	71.48	.62
16	1.0618	.3781	71.20	.34
17	1.0854	.3880	71.50	.64
18	1.1314	.4017	71.00	.14
19	1.0372	.3700	71.34	.48
20	1.0340	.3705	71.66	.80
21	1.0289	.3664	71.22	.36
22	1.0313	.3662	71.02	.16
23	1.0531	.3784	71.86	1.00
24	1.0423	.3693	70.86	.00
25	1.0397	.3682	70.82	−.04
26	1.0332	.3704	71.70	.84
27	1.0489	.3726	71.04	.18
28	1.0286	.3667	71.30	.44
29	1.0418	.3720	71.42	.56
30	1.0392	.3691	71.04	.18
31	1.0503	.3760	71.58	.72
32	1.0377	.3672	70.78	−.08
33	1.0645	.3775	70.92	.06
34	1.0320	.3680	71.32	.46
35	1.0468	.3724	71.16	.30
36	1.0268	.3649	71.08	.22
37	1.0595	.3773	71.22	.36
38	1.0574	.3768	71.26	.40
39	1.0571	.3758	71.12	.26
40	1.0462	.3718	71.08	.22
41	1.0325	.3683	71.34	.48
42	1.0363	.3695	71.32	.46
43	1.0411	.3778	71.42	.56
44	1.0391	.3701	71.24	.38
45	1.0494	.3747	71.42	.56
46	1.0314	.3673	71.22	.36
47	1.0940	.3910	71.48	.62
48	1.0637	.3779	71.06	.20
49	1.0500	.3727	71.00	.14
50	1.0211	.3630	71.10	.24

Statistical analysis of results in table 3

1. The arithmetic mean of the error = 0.3944.
2. The standard deviation of the error = 0.2284.
3. The arithmetic mean of the total solids = 71.25.
4. The standard deviation of the total solids = 0.2278.
5. The coefficient of variation of the total solids = 0.3197.
6. Four per cent of the results had a minus error.
7. The range of error of the results was from minus 0.08 to plus 1.00.
8. Thirty per cent of the results were within 0.25 per cent of the Official.
9. Seventy-six per cent of the results were within 0.50 per cent of the Official.
10. All of the results were within 1 per cent of the Official.

TABLE 4

Sweetened condensed skim milk

Official T. S. = 70.96%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.0265	.3693	71.96	1.00
2	1.0176	.3697	72.66	1.70
3	1.0274	.3695	71.92	.96
4	1.0227	.3665	71.70	.74
5	1.0384	.3702	71.70	.74
6	1.0245	.3683	71.90	.94
7	1.0292	.3700	71.90	.94
8	1.0310	.3704	71.86	.90
9	1.0202	.3676	72.06	1.10
10	1.0263	.3711	72.32	1.36
11	1.0299	.3699	71.84	.88
12	1.0359	.3741	72.22	1.26
13	1.0320	.3750	72.66	1.70
14	1.0221	.3680	72.00	1.04
15	1.0118	.3641	71.98	1.02
16	1.0327	.3728	72.20	1.24
17	1.0477	.3799	73.28	2.32
18	1.0335	.3733	72.24	1.28
19	1.0473	.3805	72.66	1.70
20	1.0224	.3766	71.74	.78
21	1.0535	.3778	71.72	.76
22	.9979	.3583	71.82	.86
23	.9965	.3569	71.64	.68
24	1.0570	.3787	71.66	.70
25	1.0377	.3731	71.92	.96
26	1.0225	.3650	71.40	.44
27	1.0386	.3715	71.54	.58
28	1.0663	.3859	72.38	1.42
29	1.0353	.3720	71.86	.90
30	1.0385	.3705	71.36	.40
31	1.0062	.3649	72.64	1.68
32	1.0573	.3813	72.12	1.16
33	1.1344	.4026	70.98	.02
34	1.0102	.3681	72.88	1.92
35	1.0207	.3706	72.58	1.62
36	1.0266	.3681	71.72	.76
37	1.0291	.3717	72.24	1.28
38	1.0253	.3745	73.06	2.10
39	1.0352	.3744	72.34	1.38
40	1.0134	.3702	73.06	2.10
41	1.0307	.3725	72.28	1.32
42	1.0296	.3732	72.50	1.54
43	1.0214	.3679	72.04	1.08
44	1.0418	.3760	72.18	1.22
45	1.0418	.3787	72.70	1.74
46	1.0394	.3813	73.40	2.44
47	1.0333	.3893	75.36	4.40
48	1.0335	.3828	74.08	3.12
49	1.0269	.3706	72.18	1.22
50	1.0294	.3677	71.44	.48

Statistical analysis of results in table 4

1. The arithmetic mean of the error = 1.2776.
2. The standard deviation of the error = 0.7214.
3. The arithmetic mean of the total solids = 72.24.
4. The standard deviation of the total solids = 0.7220.
5. The coefficient of the variation of the total solids = 0.9996.
6. No minus errors occurred in the results.
7. The range of error of the results was from 0.02 to 4.40.
8. Only one result, or 2 per cent, was within 0.25 per cent of the Official.
9. Eight per cent of the results were within 0.50 per cent of the Official.
10. Forty-two per cent of the results were within 1 per cent of the Official.
11. Fifty-eight per cent of the results varied more than 1 per cent from the Official.

Menefee and Overman (4) applied the Refractometric method to evaporated milk, and reported a standard error of estimate of 0.49.

The Simplified Vacuum Solids Test applied to ice cream mix yielded an arithmetic mean of error of 0.5090. Fisher and Watts (3) compared the Mojonnier method to the Official, and reported an average variation on the Mojonnier of 0.4830.

Sweetened condensed whole milk samples tested by the Simplified Vacuum Solids method showed an arithmetic mean of error of 0.3944. Fisher and Watts (3) tested sweetened condensed whole milk by the Mojonnier method, and reported an arithmetic mean of error of 0.57, and Menefee and Overman (4) using the Refractometric method reported an arithmetic mean of error of 0.4641.

The Simplified Vacuum Solids method results were less satisfactory on sweetened condensed skim milk than on the other products. The arithmetic mean of error was 1.2776. The Mojonnier and Refractometric methods can be applied to sweetened condensed skim milk and sweetened condensed whole milk with about equal accuracy.

The results obtained by the Simplified Vacuum Solids method compared favorably with results obtained from other methods by previous workers when the comparisons were made by statistical devices commonly used for measuring accuracy.

The Simplified Vacuum Solids Test can be performed in twenty to twenty-five minutes, which compares favorably with the time required for other common total solids tests.

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EVIDENCE FOR THE PRESENCE OF SMOOTH MUSCLE ELEMENTS SURROUNDING THE ALVEOLI OF THE MAMMARY GLAND*

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In connection with the renewed interest concerning the relation of the nerves, hormones and muscles which coordinate the important process of milk "ejection" or "letting down" at the time of milking, it seemed of interest to further investigate the type of cells surrounding the alveoli.

The literature on the subject is reviewed by Turner (1). The earlier investigators quite generally held that muscular cells or epithelial cells with contractile properties (myo-epithelial cells) were present in the subepithelial area of the alveolus. Recent observers have expressed doubt that these cells surround the alveolus directly but have suggested that the smooth muscle or myo-epithelial cells observed surround the capillaries instead.

For many years it has been known that extracts of the posterior lobe of the pituitary called "pituitrin," when injected into lactating animals cause an apparent contraction of the mammary gland and milk becomes available which previously could not be removed. (For review see Turner & Slaughter (2).) As pituitrin is considered to cause contraction of smooth muscles, especially of the uterus and blood vascular system, it seemed logical to assume that the effect of pituitrin upon the mammary gland was to produce a contraction of the muscular elements, forcing down the milk accumulated in the lumina of the alveoli and storage spaces of the duct system.

While the effect of pituitrin upon the mammary gland, uterus and vascular system has been known for a long time, only recently has evidence begun to appear indicating that it may play a role in the contraction of the udder musculature and the "letting down" of milk following the stimulation of the teats at milking time (3, 4, 5). The present concept of the relation of the nerves, posterior pituitary, adrenal and udder has been outlined (6). It should be understood, however, that positive proof is still lacking. As several phases of the problem are being investigated in our laboratory, it seemed desirable to re-examine the cellular structures surrounding the alveoli in order to determine whether muscle cells are present to aid in milk removal.

Experimental technique. The udder of a cow in advanced lactation was obtained. Blocks of tissue were placed in Bouin's fluid within an hour post mortem. The usual procedure of sectioning tissue was followed. Several

Received for publication March 13, 1941.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal series, No. 731.

stains were tried but the best results were obtained when the sections were stained first in Delafield's hematoxylin for 5 minutes, washed in water, stained 30 seconds in Van Giesen's stain, washed in water again, then passed rapidly up the graded alcohols ($\frac{1}{2}$ min. in each) cleared and mounted with clarite.

Observations. Van Giesen's stain very clearly differentiated the connective tissue by a brilliant red color. The epithelial and muscle cells were stained yellow to brown with well defined purplish brown nuclei. The smooth muscle cells were differentiated from the epithelial cells by the shape of the nuclei, the former being narrow, oblong structures while the latter were slightly ovoid or round. When the cell walls were observed, the epithelial cells were square or rectangular in shape while the muscle cells were long, narrow and more or less pointed at their ends.

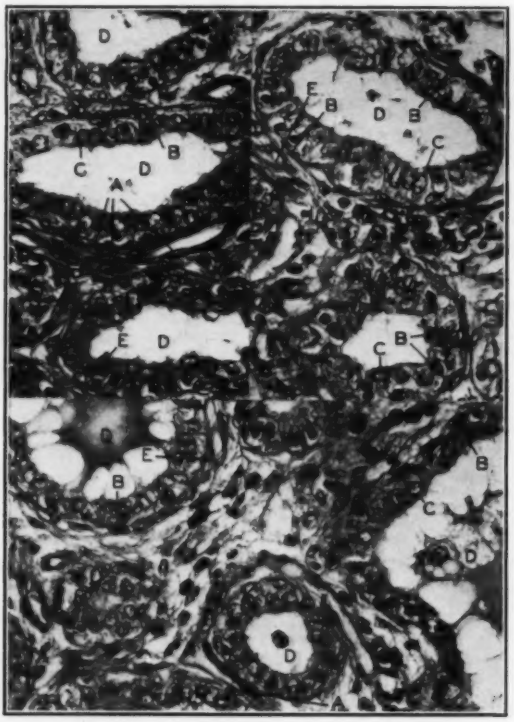

FIG. 1. Sections of mammary gland tissue from a cow in advanced lactation showing cell types of and around the alveoli. A, smooth muscle cells in the interlobular connective tissue; B, smooth muscle cells adjacent to the secretory epithelium; C, secretory epithelial cells; D, lumen of the alveolus; E, interlobular connective tissue fibers. ×375.

Occasional bundles of yellow smooth muscle cells were found scattered among the red connective tissue fibers. Individual isolated smooth muscle cells were also noticed in the interlobular connective tissue. The subalveolar cells were carefully examined. Smooth muscle cells were identified around nearly every alveolus examined (Fig. 1). At times these cells were observed in the interalveolar connective tissue, but frequently they appeared between the connective tissue membrane and the secretory epithelial cells. In no case were the smooth muscle cells observed to form a continuous band around the alveolus. Rather, they seemed to be isolated from each other and were probably of the basket cell type observed by Lenfers (7) and others.

It is difficult to determine definitely whether or not the smooth muscle cells observed directly around the alveolar epithelium are or are not associated with capillaries. However, the capillaries furnishing blood to the secretory epithelium would be of the finest calibre. As a general rule there are neither muscle fibers nor connective tissue in the walls of the true capillaries. It would appear reasonable to believe that these isolated muscle cells around the alveoli contract, thus increasing the pressure on the alveolar lumen contents and forcing the milk into the duct system. The smooth muscles around the ducts seemed to be more numerous and more distinct than those around the alveoli. Upon their contraction there would undoubtedly be a tendency to reduce the length and diameter of the ducts to aid the forward movement of the milk. While no tendency was observed for the muscle cells of the ducts to form in a circular ring or sphincter and it is not believed that muscular sphincters are present at the branching of the ducts, the present method of examination of the tissue does not entirely disprove their presence.

DISCUSSION

The commonplace act of milking sets in motion a complex series of physiological events which are just beginning to be appreciated. The removal of milk from a dairy cow is not as simple as turning a spigot on a barrel and letting the milk flow out. It is believed that the stimulus from the teats, or other sensory stimuli associated with milking, transmits to the pituitary (posterior lobe) a nerve impulse which causes a discharge of pituitrin into the blood stream. Upon reaching the udder, pituitrin causes the contraction of the muscle fibers surrounding the alveoli and ducts and forces the milk into the larger collecting spaces and cistern toward the teat. There is thus an inward squeeze upon every part of the gland which results in aiding the milker to get a greater part of the milk present.

The present study is believed to show that there are muscle cells surrounding the alveoli which upon contraction would aid in evacuating the milk from the lumen, and further that muscle cells line the fine as well as the coarse milk ducts, which upon contraction propels the milk forward to the

teat. Sphincter muscles around the milk ducts were not observed and their presence seems illogical if the above explanation of "letting down" milk is correct. The presence of duct sphincters would call for a complicated control system whereby certain smooth muscles of the ducts would be contracted while the sphincter at the same time must necessarily be relaxed.

SUMMARY

An examination of the udder of a lactating cow by histological means and suitable staining showed the presence of cells beneath the secretory epithelium of the alveoli and in the interlobular spaces which had the appearance and staining properties of smooth muscle fibers. These cells were not observed to form a continuous band around the individual alveoli but were spaced at intervals below the epithelial cells. They are believed to surround the alveoli and to aid in the expulsion of milk from the lumen and not to be associated with blood capillaries. The walls of the duct system also contained similar smooth muscle cells but no tendency was observed for these cells to form muscular sphincters which would, upon contraction, impede the flow of milk.

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VOLUME XXIV JULY, 1941 NUMBER 7

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

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Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
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THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

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JOURNALS

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Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copehagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

391. Oxidation-Reduction Potentials and the Oxidized Flavor in Homogenized Milk. P. B. LARSEN, I. A. GOULD AND G. M. TROUT, Michigan State College.

Oxidation-reduction potentials were determined both on unhomogenized and homogenized milk. In certain trials copper was added to the milk at the rate of 1 and 3 p.p.m. Homogenization was at 1500 and 2500 pounds pressure. The results showed that the Eh in both unhomogenized and homogenized milk exhibited the same trend whereas the milks showed differences in susceptibility to oxidized flavor development. Unhomogenized milk which became oxidized showed increases in potential. In contrast, the same milk when homogenized underwent similar changes in potential but showed little or no tendency to become oxidized.

392. Effect of Certain Factors Upon Lipolysis in Homogenized Raw Milk and Cream. I. A. GOULD, Michigan State College.

Fat from milk or cream homogenized at 500–1000 pounds pressure was titrated for free fatty acids. The influence of the following factors was observed: copper, salt (NaCl), formalin, storage temperature, and different fractions of milk. In addition, correlation between free fatty acids and formation of peroxides was determined. The results showed that neither copper salts in amounts as high as 10 p.p.m., nor formalin in ratio of 1:250, when added to the milk or cream, affected the speed nor extent of lipolysis. Salt markedly reduced lipolysis when added at the rate of two per cent, and almost completely prevented lipolytic activity when used at the rate of five per cent. Fat in homogenized raw milk underwent 12 fold more lipolysis at 70° F. than at 35° F., whereas the fat in the milk stored at 35° F. showed approximately twice as much hydrolysis as that in the milk held at 0° F. Pasteurization of cream and skimmilk fractions showed the active agent of lipolysis to be associated with the milk plasma. Further, precipitation of the casein by rennet and homogenizing fat in raw whey, showed that the whey exhibits definite lipolytic activity, although the major portion of the activity of skimmilk was lost with the removal of the casein. No significant relationship was observed between the extent of lipolysis in the homogenized products and the peroxide number.

393. Rancidity Studies on Mixtures of Raw and Pasteurized Milk. P. B. LARSEN, G. M. TROUT, AND I. A. GOULD. Michigan State College.

This study was conducted to ascertain under what conditions and to

what extent rancidity would occur in mixtures of homogenized and unhomogenized raw and pasteurized milk. The lipolytic changes which occurred were determined by titration of the milk and by organoleptic means. In mixtures of unhomogenized raw and homogenized pasteurized milk, acidity increases occurred with as little as one per cent of the unhomogenized milk. Increasing the percentage of the unhomogenized milk in increments up to 50 per cent increased the rate and extent of acidity development, whereas further increases resulted in a decrease in the acidity. The maximum acidity developed over a period of ten days occurred when the ratio of unhomogenized raw milk and homogenized pasteurized milk was 1:1. Rancidity was definitely detected after 7 to 10 days of storage when the mixture contained 5 to 95 per cent of the raw milk or 5 to 95 per cent of the homogenized pasteurized milk.

Mixtures of homogenized raw and homogenized pasteurized milk showed a greater acid development than did the trials in which the raw milk was unhomogenized. Further, the extent of acidity development varied directly with the amount of raw homogenized milk present, with the maximum development resulting in the sample consisting entirely of the raw milk. Development of rancid flavors in general correlated with acidity changes.

The third phase of this study consisting of mixtures of unhomogenized and homogenized raw milk gave results somewhat similar to those secured when mixtures of homogenized raw and homogenized pasteurized milk were used.

The development of rancidity in milk mixtures appears to be equally dependent upon the amount of lipase present and upon the amount of acceleration afforded by the newly created surfaces.

394. Menstruation Frequency and its Relation to Conception in Dairy Cattle. G. W. TRIMBERGER, University of Nebraska, Lincoln, Nebraska.

In two groups of 100 each for heifers and cows not bred at estrus it was found that 100 heifers and 61 cows menstruated. Two similar groups of 100 heifers and 100 cows were bred during estrus. Of the 100 heifers bred at estrus, 81 menstruated, 61 of them conceived and 52 or 85.25 per cent of those conceiving also menstruated, while of the 39 that did not conceive, 29 or 74.36 per cent menstruated. Of the 100 cows bred at estrus, 61 menstruated. Seventy-two of the cows conceived, and 50 or 69.44 per cent of those conceiving also menstruated, while of the 28 that did not conceive, 11 or 39.29 per cent menstruated.

The data do not indicate any definite relationship between breeding and conception as affecting menstruation; contrary to the general opinion among herdsmen, menstruation a few days after service is no indication that conception did not take place. From the total of 303 individuals that men-

struated, 74.26 per cent showed the menstrual discharge on the second day following estrus.

395. Cows' Urine as a Fertilizer for Bluegrass Pastures. W. B. NEVENS, Illinois Agricultural Experiment Station.

Four small plots of Kentucky bluegrass were treated in the spring months with cows' urine at rates ranging from 1250 pounds to 5000 pounds per acre at each application. A control area was untreated. Samples of the grass were harvested monthly from May to September, inclusive.

Although the urine contained more than 1 per cent nitrogen and in most cases more than 1 per cent potassium, the treatments, as a rule, were not harmful to the forage. The protein content of the grass on the treated plots was higher than that of the control area, and in most instances, the heavier the application of urine, the higher the protein content. The yields of the urine-treated plots were higher than that of the untreated pasture and there was a tendency toward higher yields from the more heavily treated plots. The palatability of the grass, as evidenced by close grazing by cattle, was higher on the urine-treated plots and the greater the protein content, the greater the intensity of grazing.

396. Butterfat and Silage Carotenoids. B. CONNOR JOHNSON, W. H. PETERSON AND H. STEENBOCK, Dept. of Biochem., Univ. of Wisconsin, Madison, Wis.

Non-carotene pigments formed by the action of acids in silage are carried over into the butter fat of the milk. By chromatographic separation the distribution of pigments in butters from cows on different forages was found to be as follows: pasture, 80 per cent carotene, 20 per cent non-carotene; molasses alfalfa silage, 68 per cent carotene, 32 per cent non-carotene; phosphoric acid alfalfa silage, 65 per cent carotene, 35 per cent non-carotene.

397. Live Weight of Cow at Various Stages of Lactation in Relation to Milk-energy Yield. W. L. GAINES, Ill. Agr. Exp. Sta., Urbana, Ill.

The problem is approached by dealing with live weight (W) at various stages of lactation and with milk-energy yield for the 8-months partial lactation (FCM). The equation, $FCM = aW^b$ is adjusted to the observations. The exponent, b, varies greatly according to the stage of lactation at which live weight is measured. FCM and W are most closely related where live weight is measured during the first month of lactation ($r = .70$). In general, the evidence of experimental observations is that within a dairy breed and within a herd FCM is proportional to the 1.07 power of live weight, where live weight is measured in the first month of lactation.

398. Effects Which Selection of Dams May Have on Sire Indexes. JAY L. LUSH, H. W. NORTON III AND FLOYD ARNOLD.

The question of what bias is introduced into the proving of sires when

the bull was mated to selected cows was investigated on 676 pairs of daughters and dams used in proving sires in Iowa D.H.I.A. records and on some 3000 pairs used in proving sires in Holstein-Friesian H.I.R. records. Only pairs where the dam had at least two records were used. The mates of each sire were divided into a high half and a low half on the basis of their first record and the regression of their later records on this first record and the regression of their daughters' records on this first record were studied. The selection of dams biases the sire proof by causing the selected dams to have breeding values above the average of the population from which they were taken but lower than the average of the records on which they were selected. This bias affects the difference between daughters and dams most severely. It affects the sire index and the daughter average about equally (the former somewhat more where each dam is represented by only one record) but in opposite directions.

That such a bias is often important in sire proving in actual practice may well be doubted, in view of what has yet been observed about how slightly the intensity of selection varies from herd to herd. The use of lifetime averages automatically tends to reduce sharply the amount of this bias but does not wholly eliminate it. In those individual cases where this bias is thought important, correction for it can be made by correcting the records on which the mates were selected toward their herd average, so as to allow for incomplete repeatability and incomplete heritability. Repeatability of differences in single records within groups of cows mated to the same sire was .43 for fat and .48 for milk in these Iowa D.H.I.A. data and was .40 for fat in the H.I.R. data. Heritability of differences between single records was .28 for fat and .33 for milk in the Iowa data and was .25 for differences between first records and .30 for differences between second records in the H.I.R. data. This is about the same as or a bit smaller than was indicated by earlier published comparisons between the daughters of high record and of low record mates of the same sires. Dominance deviations, epistatic deviations, and permanent effects of environment, all three together accounted for only about 10 to 15 per cent of the variance between records made by the mates of the same bull.

399. The Utilization of Urea by Ruminants as Influenced by the Level of Protein in the Ration. M. I. WEGNER, A. N. BOOTH, G. BOHSTEDT, AND E. B. HART, University of Wisconsin.

A basal ration consisting of corn silage, timothy hay, and a concentrate composed of ground yellow corn and ground oats, half and half (11.3 per cent protein) was fed to a Holstein heifer with a rumen fistula. Varying amounts of linseed oil meal and urea were added to the concentrate to determine the effect of the level of protein in the feed on urea utilization in the rumen. Samples of rumen ingesta were obtained via the fistula and analyzed

for protein and ammonia (urea). The protein content of rumen ingesta showed a decided increase when the level of protein in the concentrate was increased to 24 per cent. By increasing the protein in the concentrate fed to 18 per cent or more the level of protein in the rumen ingesta became greater than 12 per cent. At this point the rate of conversion of urea nitrogen to protein in the rumen began to decrease. When no linseed oil meal was added to the concentrate the added urea was utilized up to a level of 4.5 per cent (protein equivalent of 12 per cent) of the concentrate.

400. The Content of Grass-Juice Factor in Legume Silages and in Milk Produced Therefrom. B. CONNOR JOHNSON, C. A. ELVEHJEM AND W. H. PETERSON, University of Wisconsin.

Seventeen different kinds of silage were assayed for the grass-juice factor by means of their growth-promoting qualities when fed to guinea pigs on a winter milk diet. The acid preserved and soured whey treated silages were found to be somewhat higher in this factor than other types of silage.

The content of grass-juice factor of winter milk was increased by feeding silages rich in the factor, *e.g.*, silages prepared with adequate amounts of phosphoric acid.

401. Effect of Stilbestrol on the Mammary Gland of the Mouse, Rat, Rabbit, and Goat. A. A. LEWIS AND C. W. TURNER, University of Missouri.

Subcutaneous injections of stilbestrol at low dosages caused extensive duct proliferation in male mice in 2 to 4 weeks. Mammary development did not proceed farther in spayed virgin female mice similarly treated. Oral administration of stilbestrol to male mice required approximately five times as high a dosage as by injection to obtain similar results.

Castrate male rats required a higher dosage of stilbestrol than did mice to obtain mammary duct growth.

Four-tenths gamma per day of stilbestrol subcutaneously was adequate to secure extensive mammary duct development in male rabbits. After 40 to 60 days treatment early lobule development was apparent. Percutaneous administration was also effective. Mammary glands from two rabbits with subcutaneous pellets had well developed lobule-alveolar systems and responded well to lactogen treatment at 90 days. Normal females tended to lactate on stilbestrol injection.

Subcutaneous injection of stilbestrol into virgin goats caused abundant and prolonged lactation from lobule-alveolar glands. Little increase in extent of glands was apparent. Subcutaneous administration followed by pellet implantation caused no mammary gland development in a castrate male goat although the teats were hypertrophied.

BOOK REVIEW

402. Farm Animals, Their Breeding, Growth and Inheritance. JOHN HAMMOND. 199 pp., 114 fig. Longmans, Green & Co. New York, 1940. $5.00.

A brief yet reasonably up-to-date and well illustrated account of the physiology of reproduction and growth from the standpoint of what the producer of farm animals or a student just entering this field would find interesting or useful. The first two-thirds of the book is mainly concerned with such topics as time of ovulation, when to breed, artificial insemination, endocrinology as far as it affects these, differences in ages at which various parts of the body grow most rapidly, market requirements concerning weights and bodily proportions, etc. There is a separate chapter for each kind of farm livestock. The latter third of the book deals with Mendelism, evolution, selection, breeding systems and special problems in breeding for productiveness in the various kinds of livestock. J.L.L.

BACTERIOLOGY

403. Classification of the Organisms Important in Dairy Products. III. *Pseudomonas putrefaciens.* H. F. LONG AND B. W. HAMMER. Iowa Agr. Exp. Sta. Res. Bull. 285. Jan., 1941.

A technique for isolation of *Ps. putrefaciens* from water, butter and creamery equipment is described. The following special medium was developed to aid in the isolation: gelatin—4.0; proteose peptone—2.0; dipotassium phosphate—0.1; ferric ammonium citrate—0.05; agar—1.5 per cent; and water to make 100 per cent. *Ps. putrefaciens* developed readily on this medium and after several days colonies were fairly large, raised and brown to reddish brown or pink.

In certain cases enrichment in litmus milk at 3° C. followed by smearing on the special medium aided in isolation while with other materials direct smears on the medium were more frequently successful. Smears prepared with the serum of butter were more successful than smearing the butter itself. Enrichment in laboratory churnings of butter held at 3° C. also aided in certain isolations.

Ps. putrefaciens was isolated from raw and pasteurized milk, raw sweet cream, numerous samples of butter, stream, lake and roadside water in different parts of the country, private well water, city water supplies, moist soil, floors, sewers, creamery floors, churns, and various other pieces of dairy plant equipment.

Heat, acid and salt easily destroy *Ps. putrefaciens.* Pasteurization by either the flash or holder methods of pasteurization should kill any organisms of this type that might be present in the cream. Sour cream and highly ripened unsalted butter failed to yield the organism. Of 15 representative

cultures all grew in litmus milk containing 10 per cent salt, 6 in 6 per cent, 1 in 8 per cent, and none in 10 per cent salt.

In a total of 176 cultures studied in detail, variations occurred but the authors considered these too insignificant to justify varietal differences. From the standpoint of identification, the outstanding characters of the organism were found to be action on litmus milk, morphology, phosphatase production, and action on butter. P.R.E.

CHEESE

404. Western Hemisphere Cheeses Replace European Imports. ALLEN CHAFFEE, New York, N. Y. Food Indust., *13*: 3; 58–60, 107–108. 1941.

In November 1939, we imported 6,343,578 pounds of cheese as compared with 2,260,737 pounds in November 1940. Of the 1940 imports, a large amount were Argentine imitations of European types.

Because of the war, imports have stopped or nearly so, on the following cheeses: French Roquefort, all of the Italian hard-grating cheeses such as Parmesan, English Stilton, Netherlands Edam and Gouda, Danish Blue, and some of the Scandinavian goat's milk cheeses such as Gjetost.

Argentine and U. S. manufacturers are working on imitations and substitutes for the French Roquefort and Danish Blue cheeses. Opinions differ as to the quality of these U. S. and Argentine cheeses, but the manufacturers are taking it seriously and great steps forward are being made.

U. S. manufacturers have made a few substitutes for the Italian hard-grating cheeses and Argentina has made imitation Parmesan and other hard-grating cheeses. These Argentine cheeses have not been of good quality in the opinion of many importers but are improving.

Edam and Baby Gouda cheeses are past the experimental stage in the U. S. and are readily accepted by the American housewife. Methods for making trappist type cheese have been standardized but commercial production is still small.

Some of the Scandinavian cheeses are being made in the U. S. Some Swiss cheese is still being imported and American made Swiss cheese is meeting with some success. J.C.M.

405. The Keeping Qualities of Cheese and Other Milk Products. H. D. MURRAY AND A. TYRRELL. Food Mfr., *16*: 2; 39–40. 1941.

In this article the authors first give the methods used to render the milk stable and then review the production and uses of the various milk products, especially cheese, from the standpoint of keeping quality.

Milk may be rendered more stable (1) by dehydrating without altering the proportion of solids originally present, (2) by withdrawing water and

other constituents so as to yield an altered product which can be stored without elaborate precautions. In the first class are evaporated, dried and condensed whole and skimmilk, and in the second class are cream, butter and cheese.

Cheese may be made by precipitation of the curd by rennet or by acid-producing bacteria. Where rennet is used the pH is considerably higher and this results in a saving in the cheese of a greater proportion of the insoluble salts, and renders the curd capable of supporting a larger variety of micro-organisms. This last makes possible production of a greater variety of flavors, but it also makes the cheese more liable to spoilage.

A new development in the keeping of cheese is the pasteurization of the ripened cheese forming processed cheese in which micro-organism activity is almost at a standstill.

In all phases of cheese manufacture the pH is of great importance in determining the keeping quality of the product. J.C.M.

406. Bacteriology of Cheese. V. Defects of Blue (Roquefort-Type) Cheese. H. W. BRYANT AND B. W. HAMMER. Iowa Agr. Exp. Sta. Res. Bull. 283. Oct., 1940.

Black discoloration and a musty flavor in blue cheese were attributed to the growth of a mold, *Hormodendrum olivaceum*. Most of the spoilage occurred in the punch holes and cracks in the cheese. Blue cheese and cheddar cheese were made from milk inoculated with *Aerobacter aerogenes*, the organism commonly responsible for gassy defects in cheddar cheese. Only insignificant numbers of gas holes developed in the blue cheese while the cheddar cheese developed numerous gas holes. The inability of *Aerobacter aerogenes* to produce the gassy defect in blue cheese was attributed to the open texture which permitted the developed gas to escape and also to the unfavorable conditions for growth of the gas-forming organisms in the cheese.

Excessive moisture in certain parts of the cheese was shown to result in a defect characterized by a pronounced softening of the edges.

An outbreak of a defect characterized by a lack of mold growth in blue cheese was investigated. The defect was attributed to use of an atypical strain of *Penicillium roqueforti*.

An attempt was made to determine the cause of gray discoloration, a common defect of blue cheese. In cheese showing this discoloration a mousy, ammoniacal odor commonly developed and the cheese tended to become soapy with age. Two possibilities suggested for the cause of this defect were extensive growth of *P. roqueforti* and the presence of contaminating organisms. P.R.E.

407. The Cheese Industry in War-Time. JOHN G. DAVIS. Food Mfr., *15*: 8; 204–206. 1940.

This is an article discussing the making of cheese in England during this war.

It is pointed out that no better utilization of surplus milk is possible than the making of cheese, providing the whey is economically used.

The use of whey in preserving silage, as a source of lactose and for beverages are some of the ways pointed out by which nearly all the food value of the whey may be obtained. J.C.M.

DISEASE

408. Milking Machines—Their Operation and Effect Upon Milk Production and Mastitis. A. C. DAHLBERG. N. Y. Agr. Exp. Sta., Geneva, N. Y. The Assoc. Bull., Internat. Assoc. Milk Dealers, 33rd yr. No. 13; 336–340. 1941.

When cows were milked by machine in 9 to 10 minutes or in 5 minutes milk production was less persistent than when hand milking was employed. In a lactation period the final month's production in the case of hand milking was 66.7 per cent of the first month; machine milking in 9 to 10 minutes was 49.4 per cent; and machine milking in 4 to 5 minutes was 52.8 per cent. Production started to decline earlier in the case of longtime machine milking as compared with short time machine milking. It is also concluded that faster milking results in less mastitis. Favorable results were obtained by milking on a definite time schedule with a certain number of minutes for the milking period. E.F.G.

409. Disease Dissemination Through Public Sales. R. A. HENDERSHOTT, New Jersey Dept. Agr., Trenton, N. J. The Assoc. Bull., Internat. Assoc. Milk Dealers, 33rd yr., No. 13; 331–335. Feb., 1941.

The great importance of the public sale as a factor in the spread of animal diseases and the lack of sanitary supervision at sales is cited. The details of the operation of a market where qualified veterinarians under the State Veterinarian examined livestock and certified as to their health are described. The author recommends that all public sales be placed under the supervision of the agency in that state which is charged with the control of diseases of livestock. Rules are suggested which would prevent diseased animals being sold for purposes other than slaughter and going back on the farm to spread disease. E.F.G.

FEEDS AND FEEDING

410. Yield, Chemical Composition and Feeding Value for Milk Production of Alfalfa Hay Cut at Three Stages of Maturity. J. R. DAWSON, D. V. KOPLAND AND R. R. GRAVES. U. S. D. A. Tech. Bull., 739. Oct., 1940.

One each of three fields for three years was cut in the 10 per cent bloom, 50 per cent bloom and full bloom stages. In addition to total yields, chemical analysis and effect upon the stand, the feed value for milk production was determined by feeding each class of hay to three cows receiving the hay only.

There was no observable difference upon the stand. The total yield expressed as 100 per cent for the 10 per cent bloom stage was 99.4 per cent for the 50 per cent bloom and 77.7 per cent for the full bloom. The crude protein content was 18.24, 18.29 and 15.71 per cent, and the total protein per acre was 1,427, 1,381, and 977 pounds, respectively, for the three stages of cutting.

The crude fiber content was 28.86, 28.45 and 32.68 per cent, respectively, for the cuttings. T. D. N. yield per acre expressed in terms of 100 per cent for the 10 per cent bloom stage was 94.7 per cent and 70.2 per cent, respectively, for the half-bloom and full-bloom stages. The average digestion coefficients for crude fiber for the three stages were 47.7, 41.4 and 38.3 per cent, respectively. For crude protein these figures are 77.7, 77.1 and 75.4.

The daily consumption of hay was about the same, 39.4 pounds for 10 per cent bloom, 39.6 pounds for 50 per cent bloom and 38.5 pounds for full-bloom hay.

The nutrients in the earlier cut hay seemed more efficient for milk production. The calculated yield per acre of 4 per cent fat corrected milk was 6,330 pounds from the 10 per cent bloom, 5,254 pounds from the 50 per cent bloom and 3,970 pounds from the full-bloom stage. The cost per ton of hay was $7.27, $7.40 and $8.53, respectively. The calculated cost per 100 pounds, 4 per cent fat corrected milk was $.51, $.62 and $.74, respectively, for the three hays and the costs per 100 pounds of T. D. N. were $.696, $.767 and $.906. W.E.P.

411. Studies on Riboflavin and Thiamin in the Rumen Content of Cattle. C. H. HUNT, C. H. KICK, E. W. BURROUGHS, R. M. BETHKE, A. F. SCHALK AND P. GERLAUGH, Ohio Agr. Exp. Sta., Wooster, Ohio. J. Nutr. *21*: 85–92. 1941.

Bioassays of the rations and rumen contents of steers fed various rations were made for riboflavin and thiamin. The results indicated that riboflavin was synthesized in the rumen of steers fed a ration of yellow corn, alfalfa hay and a protein supplement.

The ingesta of a steer fed alfalfa alone contained less riboflavin than did the hay. The data also indicate the synthesis of thiamin in the rumen of steers. C.F.H.

412. A Quantitative Study of Vitamins in the Ruman Content of Sheep and Cows Fed Vitamin-low Diets. III. Thiamin. L. W. Mc-

ELROY AND H. GOSS, Div. Animal Husbandry, Univ. of California, Davis, Calif. J. Nutr., *10*: 163–173. 1941.

Four sheep were fed a ration containing less than 0.4 microgram of thiamin per gram. The dried rumen and reticulum contents of the sheep contained approximately 7 micrograms of thiamin per gram.

Thiamin was detected in the rumen contents of a non-fistulated cow fed a ration deficient in this aspect but not in the rumen-content of two fistulated cows. The authors conclude that their data confirm the work of other investigators that thiamin is not a dietary essential for ruminants.

C.F.H.

413. Carotene and Vitamin A in Cattle Blood Plasma with Observations on Reproduction Performance at Restricted Levels of Carotene Intake. R. E. DAVIS AND L. L. MADSEN, Bureau of Animal Industry, U. S. D. A. Washington, D. C. J. Nutr., *21*: 135–146. 1941.

The carotene and vitamin A content of the blood plasma of beef cattle was determined spectrophotometrically. The method for determination of vitamin A gave relative values because of substances in the plasma extract which cause some interfering absorption.

The animals were depleted of their reserve carotene and vitamin A before breeding. The authors concluded that vitamin A content of the blood plasma is closely related to the carotene content. Two heifers that received 60 micrograms of carotene per kilogram of body weight from dehydrated alfalfa leaf meal previous to and throughout the gestation period gave birth to apparently normal calves. Cows receiving 30 and 45 mg. of carotene produced abnormal calves. C.F.H.

FOOD VALUE OF DAIRY PRODUCTS

414. Physicochemical Assay of Vitamin A. NORRIS DEAN EMBREE, Distillation Products, Inc., Rochester, N. Y. J. Indust. Engin. Chem., Analyt. Ed., *13*: 3; 144–145. 1941.

Data are given on the stability of vitamin A when exposed in clear and brown glass bottles to daylight. Various fish liver oils and vitamin A concentrates when exposed for 5 hours in clear glass lost from 10 per cent to 55 per cent of their potency while the samples exposed to daylight in brown glass lost from 0 per cent to 7 per cent potency. The author recommends the use of brown laboratory glassware by those engaged in performing vitamin A assays on food, medicinal, and physiological preparations.

B.H.W.

415. Determination of Total Vitamin A Content of Dairy Butters. Spectrophotometric Method. R. H. NEAL, C. H. HAURAND AND F. H.

LUCKMAN, The Best Foods, Inc., Bayonne, N. J. J. Indust. Engin. Chem., Analyt. Ed., *13*: 3; 150–154. 1941.

A relatively rapid spectrophotometric method for the determination of total vitamin A content (vitamin A plus carotene) of butter is described. The fat is removed from melted butter and saponified in a solution of KOH in alcohol. The unsaponifiable matter is extracted, concentrated, and dissolved in cyclohexane. Part of the cyclohexane solution is irradiated for 1 to 1½ hours to destroy its vitamin A and carotene. Using the irradiated portion as a control, vitamin A and carotene are then determined simultaneously in the unsaponifiable extract by means of the spectrophotometer. The method was shown to yield results that were in satisfactory agreement with themselves and in reasonable agreement with the U. S. P. XI method. B.H.W.

416. The Calorie and Our Dairy Products. F. H. PLETCHER. Borden Farm Products, Brooklyn, N. Y. Milk Dealer, *30*: 5; 31, 82–85. 1941.

This article is a brief history of early research in nutrition followed by an explanation of how the caloric value of foods is determined. C.J.B.

ICE CREAM

417. Detection of Sodium Alginate in Dairy Products. CHARLES W. SCHROEDER AND PHILEAS A. RACICOT, Mass. Dept. Public Health, Boston, Mass. J. Indust. Engin. Chem., Analyt. Ed., *13*: 3; 165–166. 1941.

Since commercial sodium alginate generally contains dextrin, a quick preliminary test for dextrin may be used to establish the probable presence of alginic acid in the dairy product. To test for alginic acid, add to the sample a volume of hydrochloric acid equal to the water content of the dairy product, boil, centrifuge and repeatedly wash the solids removed by centrifuging. Dissolve as much of this residue as possible in NaOH, filter and add to the filtrate an equal volume of 95 per cent alcohol. Centrifuge and wash the solids with 75 per cent alcohol. Dry the gum so obtained and dissolve as far as possible in 0.1 N NaOH, add H_2SO_4 and let stand. If a color develops, it indicates the presence of alginic acid. By this test it is possible to detect the presence of 0.2 per cent or more of sodium alginate. B.H.W.

418. Taste, the Secret of Fountain Success. ROBERT L. SWAIN, Editor, "Drug Topics," New York. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *4*: 22. Oct., 1940.

"Taste—the Secret of Fountain Success," is the slogan used in a campaign to increase profits among the retail druggists of the country. Taste appeal should gratify all 5 senses and thus create the most favorable and lasting impression on the customer.

A survey of retail drug stores revealed that installation of a new soda fountain increased fountain business 21.7 per cent immediately; the business for the store as a whole immediately increased 14.4 per cent and eventually 20.9 per cent. The conclusion has been reached that an untapped volume of soda fountain business is available to the aggressive merchandiser.

M.J.M.

419. Working with Your Dealers. F. N. RUSSELL, Russell Creamery Co., Brainerd, Minn. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *4*: 46. Oct., 1940.

Ice cream sales can be increased by getting more accounts or by building up our present accounts. The industry is urged to spend less effort on the first and more on the second method. The advent of stores selling ice cream exclusively has shown that much more gallonage can be sold through an outlet than has been thought possible in the past. The per capita consumption of ice cream has doubled during the past 20 years but is still sufficiently low so that further sizable increases are possible.

By modernizing stores and fixtures, by planning so that the ice cream department predominates in the store, and by advertising ice cream rather than brand names, ice cream sales through present outlets can be increased.

M.J.M.

420. Merchandise and Survive. B. J. LOFTUS, Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *4*: 52. Oct., 1940.

The ultimate aim of all merchandising is to stimulate sales so that your company and the dealer will both profit. The dealers are waiting for help and the ice cream manufacturer need not keep them waiting. The author of this article advises the ice cream industry to use the material made available by the merchandising council of the Ice Cream Merchandising Institute. By making this material available to dealers an effective merchandising campaign should be the result. M.J.M.

421. Dextrose and Corn Syrup for Frozen Desserts. A. C. DAHLBERG AND E. S. PENCZEK, N. Y. Agr. Exp. Sta., Geneva, N. Y. Proc. 40th Ann. Conv. Internat. Assoc. Ice. Cream Mfrs., *2*: 31. Oct., 1940.

Three different types of corn sweeteners, dextrose, enzyme-converted corn syrup and corn syrup solids, were used in this study. The amount of sucrose

that may be replaced in ices and sherbets and ice cream was found to be about 25 per cent for best results in flavor, body and texture.

The authors state that the same relative sweetness in ice cream was secured when one pound of sucrose was replaced on a dry solids basis with 1.5 pounds of enzyme-converted corn syrup, 2 pounds of corn syrup solids and 1.1 pounds of dextrose. When 25 per cent of the sucrose was replaced on this basis, the hardness and melting rates were affected only slightly. The smoothness and richness of the ice creams were improved by the corn syrups and consumer preference slightly favored them.

A replacement of sucrose pound for pound by corn sweeteners does not give equal sweetness in ice cream, but a finished product may result which is perfectly satisfactory to the trade. The character of the sweetness is described as being different between sucrose and dextrose, with the former giving a more intense effect of shorter duration in the mouth. The question is also raised as to the desired sweetness of ice cream. During the past 20 years the sugar content of ice cream has been increased; at present most ice creams are between 14 and 16 per cent sugar. A lack of a specific percentage of sugar which satisfies most customers, and the desirability of varying the sugar content with the geographic location and the character of the ice cream itself, complicates the problem of relative sweetness of ice cream from a commercial view point. M.J.M.

422. The Application of Motion Pictures as a Medium in Showing the Influence of Several Factors Upon the Stability and Melt-Down Properties of Several Different Kinds of Ice Cream. W. H. E. REID, University of Missouri. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 101. Oct. 1940.

The application of motion picture photography has made it possible to show the relationship of several factors in the composition and manufacturing of ice cream which influence the stability and melting appearance of ice cream. These studies have been made with both black and white and colored films. The effect of a number of factors upon the melting characteristics have been determined by photographic means. M.J.M.

423. Creative Selling. DAVE COLCORD, Haskell, Oberlin Co., Marengo, Ill. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *4*: 11. 1940.

Only a small percentage of the market for ice cream has been developed. The big volume of sales lies in territories which are not using enough ice cream. Since this is so, the combined efforts of ice cream salesmen should mean more business for everybody. Time and energy do not have to be spent in trying to get competitor's accounts, but in developing new business.

The job of the salesmanager is to teach those under his direction how

to create new business. This paper is a discussion of various ways to accomplish this objective. M.J.M.

424. The Control of Overrun in Ice Cream. H. C. THOMAS, Harrington & Co., Dushare, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *3*: 39. Oct., 1940.

The ice cream consuming public and retail dealers are becoming increasingly conscious of the weight of ice cream. The production department must satisfy the salesmen and dealers by furnishing ice cream which is uniform in weight from can to can. When this is so, then a salesman can show the retailer exactly what the margin of profits will be for each can of ice cream which is dispensed.

With batch machines, overrun was controlled within reasonable limits by the use of overrun scales. When continuous freezers were installed it was then possible to control the weight of each package at the freezer. To aid in this control, scales are placed at freezers and packaging machines and the cans and packages are carefully checked as they are filled.

Reports are kept at the freezer of the unit weight of each flavor made, the number of units made, and the amounts of mix and flavorings used. Except for some shrinkage, the weights of materials used and of the finished ice cream should check. These reports, which are tabulated on monthly summary sheets, give a good record for the study and control of plant production. M.J.M.

425. Flavor Costs. J. E. SHIPLEY, Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *3*: 36. 1940.

With the exception of dairy products, the cost of flavoring materials is the largest single item in the material cost. The cost of flavoring ice cream varies from 10 per cent to 40 per cent of the total material cost, depending on the kind of flavor and quantity used.

Examples are given for arriving at the cost of 5 different ice creams.

One can change the cost of any flavor by changing any one of the following items. 1. Quantity of fruit or flavor used. 2. The percentage of butterfat in the ice cream. 3. The amount of overrun.

Public acceptance and legal standards set the minimum on flavors costs, but there are no limits set for the cost of the mix and flavoring. With this in mind it should be well worth while to check the costs on all new flavors before they are placed on sale. In this manner the introduction of an unprofitable item can be avoided. M.J.M.

426. What the Association Expense Comparisons Have Meant to Us. F. SCHWARTZ, Dairymen's League Coop. Assoc., Inc., New York,

N. Y. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *3*: 33. Oct., 1940.

The benefits which can be derived from participation in the annual expense comparisons conducted by the International Association of Ice Cream Manufacturers are discussed. The comparisons furnish a yardstick for measuring plant efficiency, distribution costs, and so on. A basis for budgeting for advertising is furnished. The comparisons can serve as a means of arriving at unit costs, and the management learns whether unit expenses for manufacturing and distribution are in line with the expenses of other companies. M.J.M.

427. Catching Up With Employee Frauds. J. S. SEIDMAN, Seidman and Seidman, New York, N. Y. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *3*: 17. Oct., 1940.

Over 200 million dollars a year are lost to industry through employee frauds. This, however, is only the amount known to be lost and does not include undetected frauds. Frequently auditing does not reveal the frauds, but they are discovered accidentally.

The nature of fraud and what can be done to eliminate it are the points stressed in this discussion. Auditing has shown sufficient plasticity and progress to justify the statement that by careful audits fraud can gradually be eliminated.

A classified outline of types of fraud reported in case studies is included in this paper. M.J.M.

428. A New Association Accounting System. O'NEAL M. JOHNSON, Statistical and Accounting Bureau, I.A.I.C.M., Washington, D. C. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *3*: 11. Oct., 1940.

The accounting system for ice cream plants, which has been developed by the statistical and accounting bureau of the International Association of Ice Cream Manufacturers, has been revised in some respects. This has been necessary because of such changes as the advent of the continuous freezer, which has eliminated much packaging machinery, newer methods of proration of service costs, more knowledge about dealer costs, and so on. The necessary changes and the suitability of the new system to meet these changed accounting conditions is explained. M.J.M.

429. Transport Refrigeration. JOHN H. REILLY. Refrig. Engin., *41*: 3; 167. 1941.

A brief description of transport refrigeration development and details of hold-over plates application. The latent heat value of the eutectic solu-

tion in the hold-over plate is approximately 106 B. T. U. per pound. The freezing point of the eutectic for low temperature work is about – 6° F. and for high temperature work approximately 18° F.

In figuring the refrigeration load for hold-over plates, two factors must be considered: (1) the necessary amount of plate surface must be used to offset the heat leakage and service load; and (2) it is necessary to have sufficient hold-over solution to absorb the heat gained in the number of hours the truck is on the route, or the length of time hold-over is desired.

Vacuumized hold-over plates result in freedom from bulging when eutectic solution is frozen and causes plates to press against coils more closely. Plates may be installed horizontally at ceiling of body, vertically on ends and sides, or may be used as partition walls. L.M.D.

430. New Developments in Ice Cream Stabilizers. B. I. MASUROVSKY. Ice Cream Trade J., *37*: 2; 37. 1941.

Domestic production of gelatine has been stimulated since the war started. Should there be a shortage of gelatine as a result of decreased importation from Europe, ice cream manufacturers will have available several other colloidal products for use as stabilizers. Among the newer types of stabilizers that are now available, are patented milk products which have some of the milk sugar removed, pectin derived from citrus fruits, cereal products of a glutenous nature, and stabilizers made from seaweeds such as kelp, and that made from other marine products such as Chondrus Crispus (Irish moss). W.H.M.

431. Streamlined Merchandising as the Dealer Will Accept It. LOUIS J. WAINER, Penn Dairies, Inc., Lancaster, Pa. Ice Cream Trade J., *37*: 2; 32. 1941.

An ice cream merchandising plan which dealers will accept must be planned carefully, followed aggressively, be simple and easy for the dealer to follow. Once such a program is put into action, consistent follow up action is needed. The effort which it takes to increase the volume of business done by a dealer is usually less than that required to get a customer away from a competitor and usually much less expensive. A survey of the locality around a dealer's store, to show him the potential volume of business which he should have, suggestions on how to improve the appearance and cleanliness of his store, and the use of the proper equipment and methods for serving ice cream, were cited as examples of things which a manufacturer might do for his dealers. W.H.M.

432. Ice Cream Production. H. BAYER, General Ice Cream Corp., Schenectady, N. Y. Ice Cream Trade J., *37*: 2; 18. 1941.

The importance of good housekeeping and sanitation are sometimes overlooked by the ice cream plant production managers, yet they are both important in the production of a quality product. A good production man must have some training in cost accounting and know when and how to purchase raw materials, how to plan production so that the best use may be made of labor. The use of motion pictures which show the various plant operations was suggested as a means of locating lost motion in plant operations. Other factors which must be watched closely by the production manager if costs are to be held down, are: securing a satisfactory yield on ice cream and novelties, efficient operation of refrigeration machines, and the avoiding of damage to equipment and possibility of accidents that may result from the use of too much water on floors. W.H.M.

433. The Manufacture of Coffee Ice Cream. B. I. MASUROVSKY. Ice Cream Trade J., *37*: 3; 51. 1941.

A brief history of the origin and source of the supply of coffee, and the composition of raw and roasted coffee is given. In testing coffee extracts to be used in ice cream the hot water test can be used. In making the test eight ounces of boiling water and 1 ounce of coffee flavoring are mixed in a glass container. The mixture is smelled and tasted and any objectional features noted. Observation on the clarity of the mixture can be made. It requires about one and one half times more coffee flavor for sherbets than for ice cream. W.H.M.

434. Menu Merchandising. ROBERT J. COOLEY. Ice Cream Trade J., *37*: 3; 16. 1941.

The importance of an attractive menu in the merchandising of ice cream is discussed. A retail store menu card successfully used by Williams—McWilliams Dairy Products, Inc., Ft. Lauderdale, Fla., is described in detail. The menu raised the gross margin of the fountain and luncheonette from 37.5 to 52 per cent by increased prices which were successfully merchandised to the consumers by means of the colorful invigorating, brilliant, oversized menu. Menus may be supplemented with printed or lettered wall signs. A large New Orleans store uses 18 x 24 inch colorful signs within frames for interior store walls. A billboard, erected on the store's parking lot may be used to call attention to some of the outstanding values on the menu. W.H.M.

435. Fitting Ice Cream in the Food and Drug Act. CHARLES M. FISTERE, Dairy Industries Committee, Washington, D. C. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 94. Oct., 1940.

A proposed regulation for foods represented for special dietary use

would put these foods, as far as label declarations are concerned, under the Food and Drug Administration. A number of regulations proposed would bring hardship to the dairy industry. It is the plan of the industry to attempt to secure clarification, or relief if necessary, when the hearing is resumed, by asking that the law be construed as not including dairy products in the category of special dietary foods.

The industry's proposal provides for a standard of identity for ice cream mix, a definition for vanilla ice cream, chocolate ice cream, fruit ice cream, nut ice cream, and finally, for ice cream containing other flavoring materials. The proposal includes a suggested definition for sherbets and for ices, thereby covering the field. M.J.M.

436. The Injection of Fruit and Syrups in Frozen Ice Cream. A. C. ROUTH, Esmond Dairy Co., Sandusky, Ohio. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 90. Oct., 1940.

After experimenting with a large number of injecting devices the following method was perfected. The method consists of metering a predetermined quantity of flavoring, by a positive pump of variable speed to the extrusion point. The next problem was to devise a means of combining the flavoring material with the ice cream. From this work a large variety of combinations has been worked out. Types of syrups and fruit mixtures which will not "bleed" out or form a surface crust were developed.

With the above experience as a background, variegated ice cream has been offered to the industry, under the trade name "Revel." Marketing experience of the first year indicates that this basic development presents an added responsibility to the benefiting industry. M.J.M.

437. Handling Labor in the Plant. LOUIS G. GALLIKER, Galliker Ice Cream Co., Johnstown, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 87. Oct., 1940.

Approximately 53 per cent of our total yearly gallonage of ice cream is made in the 5 summer months. When the employees were paid by the hour, monthly pay checks were highest in the summer when living costs were low and were lowest when living costs increased. Consequently the help was dissatisfied.

To overcome the difficulties a salary program was worked out for the plant men. The average number of hours of work during the previous year was taken as a base. The yearly salary was calculated on the basis of the number of hours and hourly rate. The men were given a yearly increase which was added to the above amount. The total figure divided by 24 gave the amount of the semi-monthly salary check. In the winter time when work is less than 40 hours a week, the difference is carried over and made

up during the summer time. Each employee receives a week's vacation with pay. When a man works after 7 p.m. he gets paid for overtime.

The employees are well satisfied under the new plan because it assures them a constant income during the winter months. M.J.M.

438. Dollars and Sense in Plant Sanitation. FRED E. YETZ, The Borden Co., New York, N. Y. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 67. Oct., 1940.

On the average the present day frozen desserts plant maintains a high degree of sanitation. It has been reported from reliable sources that the industry has progressed to the point of surpassing the milk industry in many respects.

Sanitary practices have changed materially, and a corresponding change in costs has occurred. The author believes that in many instances the added costs due to sanitary measures are not justified. An example is cited of a compact set-up of 4 freezers, three fruit injectors, and incidental piping. When all this equipment has been used it is necessary to dismantle, clean, and assemble 459 parts, 81 nuts, and 19 screws. Due to the complexity of modern machines, it has become necessary to use a higher type of employee on clean-up work than was the case some years ago. This inadvertently constituted a wise move because maintenance and replacement costs have thus been reduced.

A committee of Sanitary Control has been created within the Association. This committee is working for the adoption of reasonable and uniform standards for dairy products moving in interstate commerce. This should eliminate unnecessary expense due to lack of uniformity of requirements for inspection of methods and equipment, involving both producers and manufacturers of dairy products.

In conclusion, it was stated that a program of unquestionable and efficient sanitation calls for the allotment of a certain fixed amount of the manufacturing expense for the accomplishment of this phase of the work.

M.J.M.

439. What's New in the Dairy Industries Exposition. R. E. WRIGHT, Jersey Dairy Products Corp., Baltimore, Md. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 64. Oct., 1940.

New developments in several types of equipment were stressed, such as more efficient freezers and fruit feeders, flake ice machines, homogenizers, cup fillers and bar machines, stainless steel fittings, and delivery equipment. Sanitary construction and greater efficiency are the outstanding points of development in the new machinery specifically mentioned in the discussion.

M.J.M.

440. Factors Affecting the Viscosity and Coverage Value of Chocolate Coating for Ice Cream. J. HOFFMAN ERB, Ohio State University, Columbus, Ohio. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 52. Oct., 1940.

A number of factors determine the coverage results which will be obtained with a coating. These are as follows: 1. Composition (especially fat) of the coating. 2. Fineness of grinding. 3. Heat treatment when melting. 4. Temperature of the coating, as well as the ice cream, during the dipping operation. 5. Amount of moisture incorporated into the melted chocolate at the dipping tank. 6. The use of lecithin.

An ideal dipping arrangement was found to be somewhat as follows:

The bars were hardened in a brine tank, taken from the moulds, and dried thoroughly by chilling before dipping. The coating was melted and held in a large jacketed vat with a lid. The temperature was maintained at 115° F. by a thermostat. The coating was constantly circulated to the dipping tank and back to the vat. The viscosity of the chocolate did not increase during the dipping operation, under the above conditions.

A desirable test for controlling dipping operations is the bob test. The principle of the test is to dip a carefully weighed metal bob into the chocolate, allow it to drain and determine by weight the amount of chocolate remaining on the bob. The bob test is of great value in determining the suitability of new coatings of unknown composition. It is also very useful as a plant control test. By running bob tests at intervals, variations in the thickness of the coating due to changes in temperature, absorption of water, etc., can be detected and remedial measures can be made. Thickening of the coating due to moisture absorption is common. This can be prevented by pre-chilling the bars, which gives a better adherence between the coating and the ice cream. Coating remaining in the dipping tank from a previous day's run usually contains moisture and new coating should not be added to it. M.J.M.

441. Report of the Committee on National Standards for Ice Cream Brick Molds and Cartons. RIDGWAY KENNEDY, JR., Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 51. Oct., 1940.

Standard dimensions for ice cream brick molds and cartons were promulgated in 1936 and accepted by the industry. These standards have been found to be still satisfactory and no further changes are necessary.

The committee has studied the dimensions desirable for cartons to be used for storing ice cream in the compartments of household refrigerators. The division of simplified practice of the National Bureau of Standards has been advised of these dimensions for the carton, which have been added to

the list of U. S. Standards for Ice Cream Brick Molds and Cartons. Ice cream manufacturers have been advised as to the dimensions of this carton.

M.J.M.

442. Use of Enzyme Converted Corn Syrup in the Manufacture of Ice Cream, Sherbets and Ices. P. H. TRACY, University of Illinois, Urbana, Ill. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 47. Oct., 1940.

The recent introduction of enzyme converted corn syrup has made it desirable to study the merits of this product in ice cream, sherbets and ices. In comparison with sucrose, it depresses the freezing point of mixes somewhat more, but it has less effect in this respect than does corn sugar. It is possible to replace about one-third the sucrose with enzyme converted corn syrup, whereas, with corn sugar the replacement is usually limited to one-fourth. A one-third replacement causes a slight increase in mix viscosity and somewhat lowers the whipping ability of the mix; the flavor is unaffected but the body of ice cream is improved by the replacement.

The author states that in ices and sherbets replacements of sucrose up to 50 per cent by enzyme converted corn syrup are satisfactory. The replacements improve the body and flavor of the ices and sherbets and aid in controlling "bleeding" and surface crustation. M.J.M.

443. Vacreation of Ice Cream Mix. N. E. FABRICIUS, Iowa State College, Ames, Iowa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 23. Oct., 1940.

The practice of condensing ice cream mix in a vacuum pan has been followed for several years by some manufacturers. This procedure is believed to improve the flavor of ice cream because some off-flavors are drawn off with the vapors from vacuum treated mix. This paper presents studies made with a system of steam diffusion with vacuum treatment of ice cream mix. The machine used is called the "Vacreator" and was invented by H. Lamont Murray of Auckland, New Zealand.

In 19 comparisons of vacreated mix with vat-pasteurized ice cream mix of the same composition, an improvement in flavor score of 1.29 points was secured in the vacreated product. The treatment improves the flavor of fresh ice cream mix and the improvement is more evident the longer the ice creams are held in storage.

Vacreations of ice cream mix delayed the development of such off-flavors as stale, oxidized or metallic in the finished ice cream. The improvements in flavor score as a result of vacreation were greatest during the seasons when the fresh cream was slightly defective in flavor. This was especially true during early summer when the cream was grassy in flavor and slightly acid, and in winter when the cream was occasionally slightly rancid.

The removal of off-flavors in the ice cream made it possible to use less flavoring material and still obtain a distinctive flavor.

When using direct steam in pasteurization, it was found necessary to use water and water softeners which do not impart off-flavors to the steam. Off-flavors in the steam would be transmitted to the ice cream mix.

M.J.M.

444. The Effect of Certain Factors on the Keeping Quality of Frozen Cream. C. D. DAHLE, R. K. LAWHORN AND J. L. BARNHART, Pennsylvania State College, State College, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 7. Oct., 1940.

The main purpose of the experimental work reported in this paper was to study factors affecting the keeping quality of frozen cream containing 40 per cent fat. The cream was held for 6 to 9 months before being used in ice cream as the sole source of butterfat. From their experiments the authors draw the following conclusions:

1. The oxidation-reduction potential of cream was lowered by heat above 170° F. and was increased by copper. The Eh determined during storage is not a reliable index of the keeping quality of cream, since the Eh during storage increased greatly during the first month and then decreased to a point usually lower than the initial reading.

2. The initial Eh reading of the pasteurized cream before storage is a fair index of the keeping quality, though not entirely reliable. It was found that cream with added copper having an initial Eh of above 0.30 volts usually developed an oxidized flavor during storage of 6 to 8 months, while those below 0.30 volts seldom did. There were a few exceptions, however.

3. Cream with a cooked flavor was slower to develop an oxidized flavor. These creams had an Eh below 0.30 volts.

4. The higher temperature pasteurization (170–190° F.) resulted in creams of superior keeping quality when copper was present in abnormal amounts.

5. A pasteurizing temperature of 150° F. for 30 minutes offered no protection during storage if the copper content exceeded 0.6 p.p.m. A temperature of 170° F. flash offered protection in creams having a copper content over 0.75 p.p.m. but under 1.12 p.p.m., while 190° F. flash was only slightly better than 170° F.

6. Oat flour to the extent of two per cent of the fat present offered protection with over one p.p.m. of added copper.

7. Sugar to the extent of 10 and 15 per cent of the cream aided defrosting and reduced "oiling off," but did not improve flavor.

8. Reducing the acidity of frozen cream ice cream mixes to 0.10 per cent practically restored the normal whipping ability.

The paper contains an excellent summary and review of previous published material about the storage of frozen cream and its use as an ingredient of ice cream. M.J.M.

445. The Use of Corn Syrup Solids in Ice Cream and Ices. L. R. GLAZIER AND M. J. MACK, Massachusetts State College, Amherst, Mass. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 40. Oct., 1940.

In this study the effect of partial replacements of corn syrup solids for sucrose on the flavor, body and textures and other properties of ice cream, has been observed. A slight majority of consumers failed to notice a decrease in sweetness caused by the replacement of 20 and 26⅔ per cent of the sucrose of the mix by corn syrup solids. However, the replacement improved the body and texture in the opinion of about 70 per cent of the people, and the majority preferred the ice cream containing the two sweeteners. Since the most obvious difference between the samples was in body texture, the judges apparently expressed a final preference for the ice cream with the best body and texture.

The results show that sugars have two principal functions in frozen dairy products. One is to contribute sweetness and the other is to improve body and texture. Though sucrose is somewhat sweeter than the corn sweeteners certain combinations of sucrose and corn sweeteners improve body and texture more than sucrose alone. Both of these factors should be considered by the ice cream manufacturer.

Partial replacements of sucrose by corn syrup solids were found to have very little effect on titratable acidity and protein stability, to raise the freezing point slightly, to increase the mix viscosity about 10 per cent, and to affect only slightly the whipping ability of the mixes. The development of sandiness and extreme coarseness, during prolonged storage of ice cream, is delayed by the use of corn syrup solids.

A combination of sucrose and corn syrup solids improved the body and texture of ices and sherbets, as well as ice bars. The change in body and texture produced by the combination of these sugars is more marked in ices and sherbets than in ice cream. M.J.M.

446. Detection of Sodium Alginate in Dairy Products. ANONYMOUS. Food Mfr., *15*: 11; 265. 1940.

Sodium alginate may be detected in milk, cream, cream cheese and ice cream by the following method: Boil sample with 6N HCl and wash residue first with 75 per cent alcohol and then with ether until free of soluble matter. Dissolve in dilute NaOH and filter. Precipitate the gum from the filtrate by adding an equal volume of alcohol and wash free of soluble matter with 75 per cent alcohol. Dissolve the purified dried residue in 0.15 cc. of tenth normal NaOH and add 1 cc. of concentrated sulphuric acid saturated with $Fe_2(SO_4)_3$. In the presence of sodium alginate, a purple color develops after standing.

The weight of residue sufficient to give the color test is 0.1 mg. and

starch, gelatin, Irish moss, agar, gum arabic, formaldehyde, etc., do not interfere with the test. J.C.M.

MILK

447. Factors Influencing the Flavor of Milk. P. F. SHARP, Cornell Univ., Ithaca, N. Y. Dairy World, *19*: 11; 28. April, 1941.

The author presents a rather complete summary of the subject of milk flavors and discusses causes and remedies for the six types of flavor changes: Microbial growth, feed, absorbed, chemical composition, processing and handling and enzymatic and catalytic. F.J.D.

448. Six Day Delivery from the Plant Angle. H. D. DRAIN, Peoples Dairy Co., Akron, Ohio. Dairy World, *19*: 11; 17. April, 1941.

A brief discussion of the plant problems involved in a six day delivery system for fluid milk. The main difficulties involve supplying 40 to 60 per cent more milk for delivery the day before and the day after the day deliveries are omitted; supply and storage of milk; bottles and cases; handling returns; and arrangement of plant working schedules. The author states that in his plant a saving of one and one-half men has been realized and customers have cooperated very satisfactorily. F.J.D.

449. Frozen Milk. ANONYMOUS. Food Mfr., *16*: 2; 42. 1941.

This is an account of studies made to determine the effect of reconstituted, concentrated, frozen whole milk of (a) degree of concentration, (b) homogenization, (c) length of holding period, (d) temperature of thawing.

Fresh whole milk was pasteurized at 143° F. for 30 minutes, condensed in a small vacuum pan, placed in 2-quart cans, and set in a refrigerator having an average temperature of −10° F. until removed for examination. The homogenized samples were processed at 2,000 lbs. following pasteurization and preceding concentration.

The samples were tested for: flavor, organoleptically; per cent of acidity by titration; creaming ability by allowing to stand in 100 ml. cylinders for 24 hours at 40° F; de-emulsified fat by a method similar to that of Webb and Hall, except that Babcock test bottles were used and the samples were examined after reconstitution; stability in boiling water by adding a few drops of sample to boiling water in a test tube; and appearance of fat separation by visual examination.

The data obtained indicated that milk may be concentrated and held at low temperatures for several weeks without greatly changing its characteristics and quality. A concentration of 3 to 1 appeared to give best results. Tallowy flavor was noted in most of the held samples.

Homogenization gave a final reconstituted product that was smoother in

texture and exhibited less de-emulsified fat. The creaming ability of the milk was completely destroyed and apparently homogenization inhibits development of tallowy flavor.

Curd tension is reduced by each of the processing steps and the two samples of homogenized reconstituted milk tested 0 and 7 grams, while the original milk tested 50 and 47 grams respectively. J.C.M.

450. Milk Production Trends. JOHN L. WILSON, Agr. Marketing Service, Washington, D. C. The Assoc. Bull., Internat. Assoc. Milk Dealers, 33rd yr., No. 13; 317–330. Feb., 1941.

With many charts and much factual material the author presents a picture of rather heavy production and good demand during the summer and fall of 1940 with a prospect of low feed costs and increasing numbers of dairy cattle furnishing a heavier supply of milk during the next year or two at least. E.F.G.

451. A Critical Review of the Phosphatase Test. L. H. BURGWALD, Ohio State Univ., Columbus, Ohio. The Assoc. Bull. Internat. Assoc. Milk Dealers, 33rd yr., No. 14; 366–389. Feb., 1941.

The test is considered to be accurate for both the holding method and the short time method of pasteurization of milk. It is not quite as accurate for cream and sometimes tests change from negative to positive as the cream ages. Higher temperatures of pasteurization are needed to secure negative tests with ice cream and chocolate milk. Little work has been reported on cheese but these reports indicate it can be applied with a certain degree of accuracy. In the case of butter it appears as if further research is necessary.

This is a very comprehensive review article and has a list of fifty-five references. E.F.G.

452. A Study of Milk Cooling Systems for the Farm. F. C. FENTON, Kansas State College, Manhattan, Kans. The Assoc. Bull., Internat. Assoc. Milk Dealers, 33rd yr., No. 13; 341–357. Feb., 1941.

Reports on cooling practices were obtained from 1,144 dairymen in several larger Kansas milk sheds and a detailed study of costs and methods was carried out on the farms of 60 large producers. Of the 76 per cent using some cooling method 40 per cent used well water, 12 per cent used ice and 24 per cent used mechanical coolers of which 28 per cent was wet storage, 48 per cent dry box and 24 per cent walk-in boxes. Of the 60 larger producers surveyed 66 per cent used mechanical coolers of which 44 per cent were wet storage, 30 per cent dry boxes and 26 per cent walk-in units. Well water was used by 21 per cent and ice by 13 per cent. Wet storage units are

typically used by producers who deliver to pasteurizing plants and dry storages by producer distributors.

Milk in 10 gallon cans in dry storage at 41° F. was above 70° F. after 8 hours indicating unsatisfactory cooling. When cooling with 62° F. well water, pumping the water at the rate of 5 gallons per minute, the milk temperature was lowered to 71° F. in 1 hour. Continuous agitation of the milk lowered the temperature to within 2 degrees of the cooling water in 1 hour. With the mechanical wet storage units with 35° F. water agitation of either the milk or water is necessary to reach 50° F. in 13 minutes. If an ice bank is used, a smaller tank will be satisfactory. Complete records were kept for one year on 24 farms. Current consumption per 100 lbs. of milk during four summer months averaged slightly over 2 k.w. hours for both wet and dry storage with the cost of the latter slightly greater. With current at 3 cents per k.w. and ice at 30 cents per 100 lbs. total costs per 100 lbs. for wet storage were 9 cents, dry storage 2.7 cents and ice 40.6 cents. E.F.G.

453. A Study of the Time Temperature Relationships in the Pasteurization of Milk as Regards Creaming, Phosphatase and Bacterial Destruction. F. R. Holland and A. C. Dahlberg, N. Y. Agr. Exp. Sta., Geneva, N. Y. The Assoc. Bull., Internat. Assoc. Milk Dealers., 33rd yr., No. 14; 361–365. 1941.

On a small laboratory scale the effect of pasteurization upon creaming ability, phosphatase test and bacterial efficiency were studied at 5° intervals between 140° F. and 175° F.; also including 143° F., 162.5° F., and 172.5° F. It is concluded that the tests to which milk is subjected for the determination of efficiency of pasteurization are too severe for the present standards when used in conjunction with very brief heating periods. It was found that the temperature of 170° F. could be held for 2½ seconds without destruction of cream volume and also at this temperature *Escherichia coli* and phosphatase were inactivated without a holding period at all. It is suggested that it may be possible to obtain the desirable effects of pasteurization at 170° F. for 1 second and at the same time secure a much less cooked flavor than at 143° F. for 30 minutes. E.F.G.

454. Flavors in Milk. John G. Davis. Food Mfr., *15*: 11; 272–275. 1940.

This is an exhaustive review of milk flavors, their cause and occurrence.

Milk flavors may be divided into normal and abnormal. This latter division then breaks down into classifications as physiological, such as feed flavors and excessive cowiness; enzymic, *i.e.*, rancidity; chemical, such as oxidized and cooked flavors; bacterial, which includes souring and fruity flavors; and lastly, mechanical acquired flavors such as chlorine and soap flavors.

Physiological flavors are caused by the feed or pasture; enzymical flavor causes are not very well understood. Chemical change is caused by copper contamination, overheating, etc.; bacterial, by improper care of the milk; and mechanical by contact with foreign substances. J.C.M.

PHYSIOLOGY

455. The Effect of Refeeding and of the Administration of a Pituitary Extract on the Ovaries of Undernourished Guinea Pigs. D. J. STEPHENS AND WILLARD M. ALLEN. Endocrinology, *28*: 580. 1941.

Undernutrition in female guinea pigs of sufficient degree to cause a loss of 20–30 per cent of body weight in a period of 2 weeks resulted in atrophic and retrogressive changes in ovaries similar in character and degree to those following hypophysectomy. These changes were not affected by the administration of vitamin supplements but normal ovarian structure was restored by refeeding. The ovary of the undernourished guinea pig remained responsive to stimulation by at least one of the hypophyseal gonadotrophic hormones. These results were considered to support the contention that changes occurring in the ovary during inanition may be due, at least in part, to the inability of the anterior pituitary to continue to produce sufficient gonadotrophic hormone to maintain the normal structure and function of the ovary when the food intake is markedly curtailed. R.P.R.

456. Relation of Nutrition to Mammary Growth after Estradiol Administration to Hypophysectomized Rats. L. T. SAMUELS, R. M. REINECKE AND W. E. PETERSEN. Proc. Soc. Exp. Biol. and Med., *46*: 379. 1941.

Well nourished by stomach tube feeding, adult virgin hypophysectomized rats did not show mammary development following the administration of 1,000 I.U. of estradiol benzoate every other day for 28 days.

457. Influence of Local Applications of Turpentine on Mammary Gland Growth and Involution. J. P. MIXNER AND C. W. TURNER. Proc. Soc. Exp. Biol. and Med., *46*: 437. 1941.

The application of spirits of turpentine for 7 days to the teats and adjoining skin of lactating mice weaned on the 4th day after parturition retarded the rate of involution of the mammary lobule-alveolar systems. Similar applications to spayed and normal females failed to stimulate alveolar growth and pseudo-pregnancy was not produced in the latter group. R.P.R.

458. The Relation between the Fat and Carbohydrate Metabolism of Lactation, as Indicated by the Respiratory Quotient of the Mammary Gland. E. P. REINEKE, W. D. STONECIPHER AND C. W. TURNER, Mo. Agr. Exp. Sta. Am. J. Physiol., *132*: 535–541. 1941.

The average respiratory quotient of the active mammary gland in 20 experiments on normal unanesthetized goats was 1.17 ± 0.036. Local anesthetization (apothesine) in 15 experiments resulted in a mean respiratory quotient of 1.15 ± 0.034. Use of nembutal as a general anesthetic during the sampling of blood resulted in a mean respiratory quotient of 1.09 ± 0.0115. Correction for the synthesis of urea in the mammary gland (according to Graham) would increase this figure to approximately 1.18.

The respiratory quotient of the mammary gland of the non-lactating-pregnant goat during the last half of pregnancy was found to be identical with that of the lactating goat, while that of the non-lactating non-pregnant goat had declined to 0.87. Apparently, the increased metabolic requirements of the gland during lactation are met by an increase in the rate of blood flow.

The mammary gland of fasted goats showed a decline in the respiratory quotient to around 0.80, a figure comparable to that reported by other workers for the perfused excised udder.

Because of the parallel between the decline of the respiratory quotient of the mammary gland and the known decrease in the lower fatty acids of milk during fasting, it is suggested that the synthesis of milk from carbohydrate is confined largely to the fatty acids of low molecular weight.

D.E.

459. Androsterone Effect on Pituitary and Mammary Gland. R. P. REECE, New Jersey Agr. Exp. Sta. Proc. Soc. Exp. Biol. and Med., *46*: 265. 1941.

Thirty sexually mature rats were ovariectomized and paired on the basis of body weight. One of each pair received subcutaneously daily for 15 days 200 gamma of androsterone. The other one of each pair received no treatment. The hormonal treatment caused no significant change either in pituitary weight or pituitary lactogen content and produced no detectable growth of the mammary glands.

R.P.R.

460. Secretion of Orally Administered Radio-Iron in the Milk of Cows. L. A. ERF, Crocker Radiation Lab., University of California. Proc. Soc. Exp. Biol. and Med., *46*: 284. 1941.

Radio-active iron was converted into an iron chloride solution and 115 cc. of this solution administered (by drenching) to 2 cows. One of the cows had previously received 8 gm. of ferrous sulphate twice daily for 12 days. One

of the cows produced 13,350 cc. of milk and secreted an average of .121 per cent of radio-iron per liter of milk, or a total of 1.5 per cent of the amount administered, during the 78-hour period. The second cow (received ferrous sulphate prior to the administration of radio-iron) produced 18,810 cc. and secreted .128 per cent per liter, or a total of 2.5 per cent of the radio-iron administered. It was suggested that "If the average cow producing 4 liters of milk a day can secrete in the milk 0.5 per cent of a 10 gm. oral dose of iron per 24 hours, 12.5 mg. of iron would be present in each liter—an amount more than adequate for a growing child who consumed a liter a day." R.P.R.

461. An A. C. Induction Flow Meter for Measurement of Blood Flow in Intact Blood Vessels. A. KOLIN, Int. Sinai Hospital, New York City. Proc. Soc. Exp. Biol. and Med., *46*: 235. 1941.

A modification of the electro-magnetic flow meter was presented. The modification employs an electro-magnet fed by an alternating current. This eliminates disturbances due to polarization and variable galvanic potentials and makes it possible to use an alternating current amplifier. R.P.R.

MISCELLANEOUS

462. Cooperative Advertising. D. T. CARLSON, President, Am. Dairy Assoc., Willmar, Minn. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *4*: 30. Oct., 1940.

The American Dairy Association began its activities in January, 1940. Its purpose is to organize and consolidate on a national basis all state and local agencies for promoting the consumption of dairy products. This is not a duplication of the activities of the National Dairy Council, which are largely educational in nature. The American Dairy Association will carry on commercial advertising of dairy products.

The plan of coverage to be undertaken by the association is advertising through newspapers, radio spots and point-of-sale merchandising in selective markets. The newspaper coverage is now five and one-half millions. Heretofore, advertising has largely emanated from processors through the advertising of specific brands. That type of advertising may succeed in transferring demand from one brand to another but is not effective in increasing *per capita* consumption. By effective cooperative advertising of dairy products the *per capita* consumption of these foods can be increased. M.J.M.

463. Merchandising as the Dealer Will Accept It. LOUIS J. WAINER, Penn. Dairies, Inc., Lancaster, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *4*: 37. Oct., 1940.

There are no new and revolutionary principles in merchandising; profitable merchandising therefore consists of putting into practice the things known to be effective. If dealers are to accept one's merchandising plans, these four things must be considered: 1. A carefully planned program; 2. An aggressive program; 3. A simple program which the dealers can easily follow, and 4. The program must have a consistent follow-up plan. A merchandising plan will appeal to dealers when they can see that it will be profitable to them, as well as to the ice cream company.

An effective merchandising plan is the cheapest way to increase gallonage. Increased sales through existing outlets can be realized with less expense than by securing new outlets. M.J.M.

464. Dusinberre and Oaks Case. HARRY POLIKOFF. New York Metropolitan Milk Marketing Area. Milk Dealer, *30*: 5; 41–44. Feb., 1941.

A discussion is given of the Dusinberre and Oaks case, in which the New York court of appeals sustained the statutory authority of a State control agency to deny a milk dealer's license where no violation of law had been shown, and no intention or attempt to violate. The applicants' lack of experience and "doubtful" prospects were deemed as tending toward destructive competition in a market found to be adequately served and stable without them. C.J.B.

465. Care and Maintenance of Walls. L. C. THOMSEN, Dairy Dept., Univ. of Wis. Milk Dealer, *30*: 5; 36, 72–74. Feb., 1941.

The importance of light in the dairy plant is stressed. A table is given which shows the percentage of light reflected by colored paints. Other factors discussed are: Dampness and mold growth on walls and ceilings; discoloration of paint; peeling of paint; and tile or glazed brick walls. C.J.B.

466. Consumer Friendship. RACHAEL REED, The Borden Co., Chicago, Ill. Milk Dealer, *30*: 5; 34, 68–71. Feb., 1941.

A discussion is given to the importance of consumer friendship and how it can be improved through understanding. It is pointed out that there are factors peculiar to the entire dairy industry, at least two of which are complex and require detailed study to be understood. They are: 1. The inter-relation between cost of basic product milk for fluid use and cost of the product for manufacturing purposes. 2. The inter-relation between production costs in different parts of the country. The author believes that the consumer attitude toward price is an educational problem. Other methods of improving consumer friendship are discussed under the head-

ings: "Do not take consumers by surprise," and "Relations with Employees." C.J.B.

467. Ice at Lower Cost. ANONYMOUS. Milk Dealer, *30*: 5; 32, 49. Feb., 1941.

A discussion is given of how some milk dealers are obtaining ice at a lower cost by replacing block ice plants with large-size ice-producing machines. C.J.B.

468. Accounting in 1941. FRED PRESTON, The Borden Co., Columbus, Ohio. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *3*: 7. Oct., 1940.

The increasing need for the accountant is stressed. Fixed costs are at present rising, hence careful accounting is essential so as to control costs as much as possible. The National preparedness program doubtless will affect plant operations. The added costs of additional taxes, higher labor costs, higher material costs, increased costs of new machinery, trucks, and so on, will make it difficult to cover these added expenses through additional revenues. If this happens, then the only alternative is that of more economical operations. M.J.M.

469. The Human Side of Advertising. H. K. DUGDALE, Van Sant, Dugdale and Co., Inc. Ice Cream Trade J., *37*: 3; 35. 1941.

An extensive psychological study conducted by one of our leading universities has shown that there are seven basic buying motives or seven primary appeals which if properly used are the most likely to induce people to do what we want them to do. They are: 1. Self-preservation; 2. Profit or love of gain; 3. Appeal to pride or vanity; 4. Appeal to family affection; 5. Civic pride and patriotism; 6. Utility appeal, and 7. Taste. Instead of telling you what a product is and how it works, you are told by advertising what it will do for you, what it has done for other people, why you cannot get along without it, where you can get it, and before you know it you are doing exactly what you were asked to do. W.H.M.

470. Refrigeration Drives. E. C. LINDSAY, JR. Refrig. Engin., *41*: 3: 173. 1941.

A brief resume of types of mechanical drives used generally in the refrigeration industry to transmit power from the prime mover to the driven machine. They being as follows: 1. Direct connected motors, with or without flexible couplings. 2. Gear drives, including reducers. 3. Chain drives. 4. Flat belt drives. 5. V-Belt drives. L.M.D.

471. Keep Employees Safe. EDGAR G. QUESNEL. Ice Cream Trade J., *37*: 3; 30. 1941.

Most accidents that have occurred in dairy plants could have been prevented. Dissatisfaction, poor physical condition, lack of interest, lack of consideration for others, acquired or permitted unsafe practices or work habits are the principal reasons which cause accidents. These conditions can be corrected if management can in some way get closer to men and develop a mutual understanding. This can best be done through the medium of interested personal contact. W.H.M.

472. Increasing Production Per Kilowatt Consumed. R. E. MILLER, York Ice Cream Machinery Corp., York, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., *2*: 74. Oct., 1940.

Some of the features of design and operation tending toward high refrigeration costs are discussed and corrective measures are cited. High refrigeration costs are avoidable, hence a thorough knowledge of their causes and means of prevention are essential.

Corrective measures in one plant may not be suitable in another. Each situation must be carefully studied from the standpoint of its special requirements. Production schedules, power and water rates, and so on, are variables. The only way to determine whether the most is being obtained from refrigeration equipment is by a plant survey conducted by a person competent in solving refrigeration problems. M.J.M.

473. The National Institute for Research in Dairying. T. CROSBIE-WALSH, Food Mfr., *15*: 11; 268–271. 1940.

This article describes the National Institute for Research in Dairying and gives some of the work done by it.

The Institute is made up of four main divisions. These are Dairy Husbandry, Chemistry, Bacteriology, and Physiology and Biochemistry. The Experimental Dairy and Library are separate departments though much smaller.

The Institute carries on studies such as the nutritive value of milk and milk products, pasteurization methods and methods of milk analysis. In addition dairy equipment is tested at the Institute. J.C.M.

474. War-Time Packaging. ANONYMOUS. Food Mfr., *15*: 12; 292–295. 1940.

This article reviews the field of new packages, new linings and prospective changes in food packaging.

Kraft Cheese Co. introduced a thrift package, a spiral-wound cardboard tube with a slip-over lid. Another Kraft package dispensed with the lid,

covering the cheese with a transparent film over a shallow cardboard tray.

At the start of the war, waxed paper containers were already beginning to be used as "one-trip" receptacles for milk, boiled sweets, etc. The war restricts the use of these containers in the milk and cream industries but greatly promotes their use in other food industries.

A new material called W. 535 has been brought out by Imperial Chemical Industries. This material is used to replace paraffin wax or to increase the usefulness of the paraffin. Due to smaller crystals and voids, the permeability of W. 535 is much less than that of the paraffin wax and when folded a water-permeable crack does not develop. J.C.M.

475. The Thermal Insulating Properties of Silica Aerogel. JOHN F. WHITE. Refrig. Engin., *41*: 3; 171. 1941.

A comparatively recent addition to the list of materials being offered to American industry. Silica aerogel is the dried gel obtained by dehydrating silica jelly in such a manner that the solid colloidal structure present in the jelly undergoes no change. It is a light, voluminous solid having a density of 7.0 lb. per cu. ft. and a pore volume of about 94 per cent. Its thermal conductivity is approximately 10 per cent less than that of still air. If the material is opacified its insulating value is enhanced. It may be heated to 1,400° F. without undergoing any change. Very little moisture absorption occurs. Unopacified aerogel will pack to 7.5 lbs. per cu. ft. and the opacified 8.25 lb. per cu. ft. It is particularly suited for cabinet wall insulation, because due to its efficiency, it can be used to decrease wall volume, thus increasing storage capacity within the same over-all dimensions. On the other hand, if used in same thickness as other insulating material a 50 per cent reduction in refrigeration may be made. It is ideal for low temperature insulation. Research is in progress leading to commercial production of a grade that has a density in the order of 3.75 lb. per cu. ft. L.M.D.

476. Moisture, Its Sources, Effects and Control. R. L. HOCKLEY. Refrig. Engin., *46*: 3; 179. 1941.

The author lists freeze ups, the formation of sludge, hydrolizing of the refrigerants to form acids, and copper plating as the difficulties resulting from moisture in a refrigeration system. He describes the various methods of installing dehydrators, and the two general types of dehydrating agents, chemical and physical. L.M.D.

477. A Public Relations Program Geared to 1941 Needs. HAROLD W. COMFORT, Borden Co. Milk Dealer, *30*: 5; 112–119. Feb., 1941.

The reasons for some of the opinions regarding the milk business which are now entertained by the public are given and means of correcting these

opinions are discussed. The author concludes his discussion as follows: "In developing better public relations, we should always keep the fundamentals in mind lest we lose the substance by grasping at shadows. Let us continue developing sound operating policies to meet changing conditions; let us put our house in order—if corrective measures are needed. And then let us demonstrate conclusively to all the people who make up our various publics that this industry operates in complete harmony with public interest."

C.J.B.

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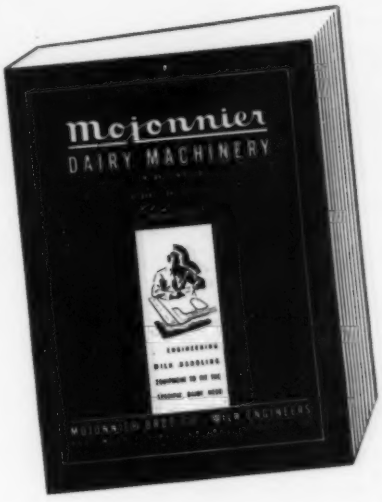

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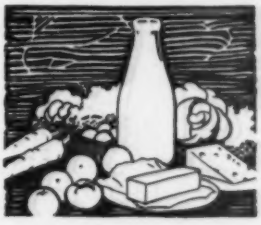

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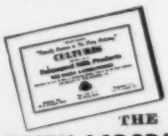

THE
DAIRY LABORATORIES
DIVISION DALARE ASSOCIATES

Dept. J-71, Phila., Pa.
BRANCHES
New York Baltimore Washington
See our catalog in Dairy Industries Catalog.

NOW . .

Your Enemy!

MOLD MYCELIA!

LET MODERN SCIENCE PROTECT YOU!

A Complete Parsons Official Mold Mycelia Test Kit with Highest Grade Equipment, and enough Chemicals and Supplies with simple "Can't-Go-Wrong" directions to make

1,000 Mold Tests . . . $10.00
2,000 Tests, $14.50; more Tests, lower cost each.
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EVERYTHING YOU NEED FOR MOLD MYCELIA AND SEDIMENT TESTING

Exclusive distributors for

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SEDIMENT TESTING SUPPLY CO.

K-20 E. JACKSON BLVD.,
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SOLVAY CLEANSER #300

Used by dairies and creameries the country over. Cleanser No. 300 has an excellent reputation for giving maximum effectiveness as a general utility cleanser. HERE ARE THE ADVANTAGES YOU GET BY USING CLEANSER No. 300:

1. Not harsh on the hands.
2. Cleans *clean* under a wide variety of dairy conditions.
3. Cleans with minimum effort.
4. Harmless when used properly on such materials as painted surfaces and metal equipment.
5. Effective under practically all water conditions.
6. Rinses easily.
7. Uniform composition produces dependable results.
8. A little does a good job.

WRITE TODAY FOR COMPLETE FOLDER!

SOLVAY CLEANSER #400

This cleanser is slightly more active than Number 300. It is particularly advantageous where exceptionally dirty equipment is to be cleansed. It has gained wide popularity in plants as a cleanser of medium strength.

WRITE FOR COMPLETE FOLDER!

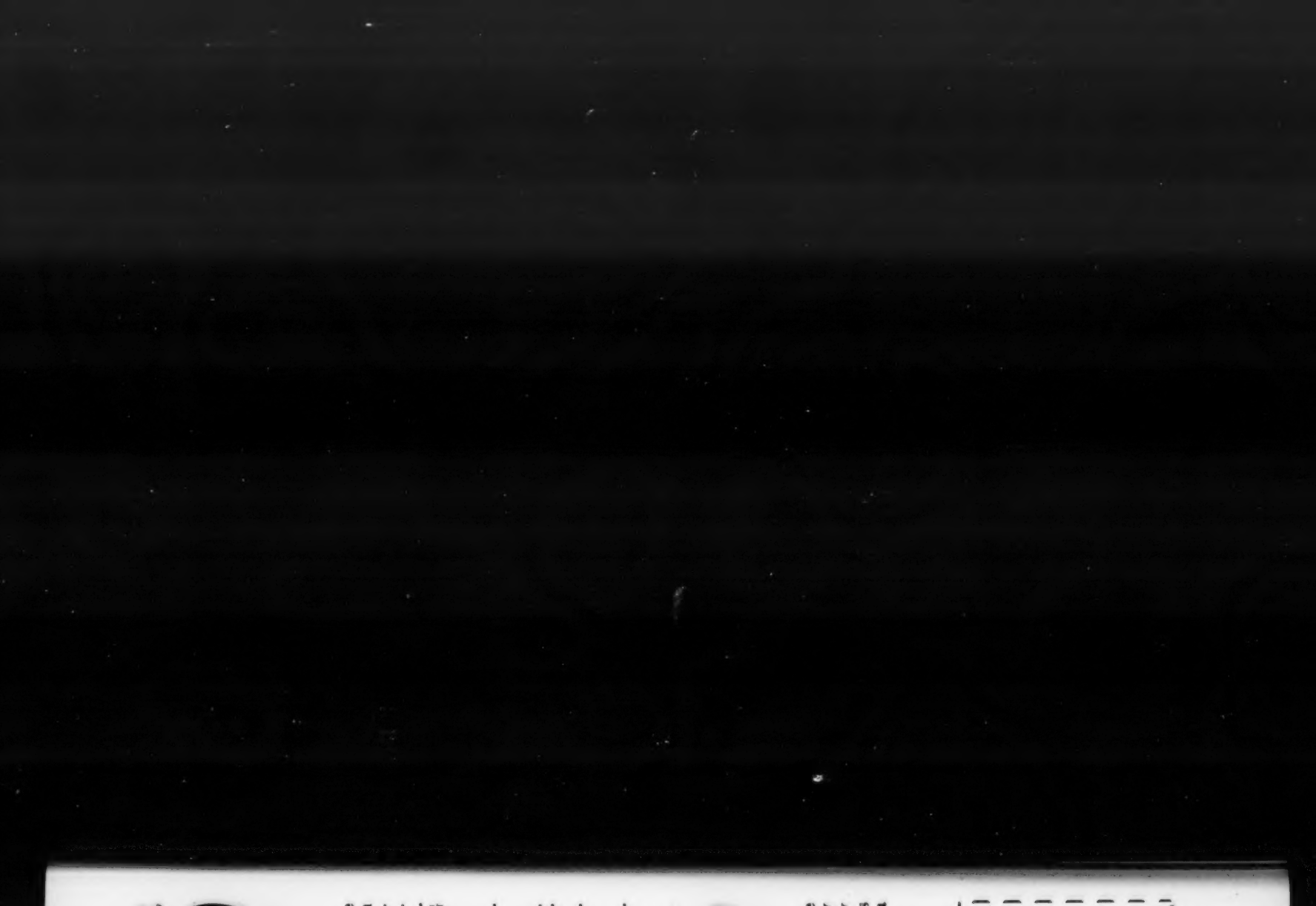
60th ANNIVERSARY 1881 • 1941

SOLVAY SALES CORPORATION
40 Rector Street, New York, N. Y.

☐ Please send me your complete folder on Cleanser No. 300
☐ Please send me your complete folder on Cleanser No. 400

Name .....
Associated With .....
Address .....
City ..... *State* CX-741

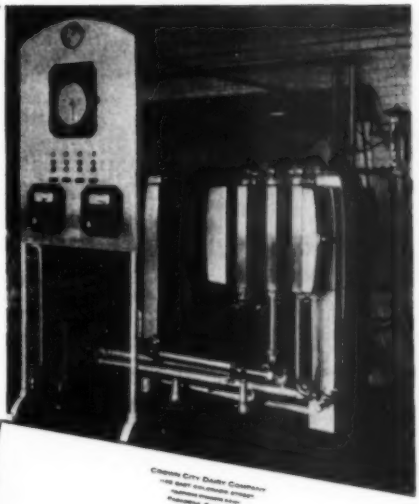
Your advertisement is being read in every State and in 25 Foreign Countries



Taste Test

STUMPS THE EXPERTS

Crown City Dairy Demonstrates That Milk Pasteurized with CP FULL-FLO PLATE H.T.S.T. Equipment Retains Original Flavor



Mr. A. G. Marcus,
President
Crown City Dairy Co.
Pasadena, Calif.

June 12, 1941

Mr. A. J. Cowell, Manager,
Creamery Package Mfg. Co.,
7905 East 11th Street,
Los Angeles, California.

Dear Mr. Cowell:

We have had a very interesting experience with our new system of Short Time High Temperature method of pasteurizing milk. First, let me say that your installation men were very considerate in their work, interfering as little as possible in their normal routine of our daily work, and the way they completed their installation of new equipment so that we had little or no trouble. Our CPFF pasteurizing equipment, being the first of its kind in the East, received an unusual amount of examination and inspection by public officials and others, and I am pleased to report that the equipment, as well as the finished product, has been approved, and we have had very nice compliments from all concerned.

The equipment is operating efficiently, is much quicker than our old method of pasteurizing, and for many reasons is much more economical. All bacteria counts have been uniformly good. There has been a very noticeable improvement in the flavor of our pasteurized milk, as compared with that which we had previously, and that of our competitors. In this connection, we have had many fine compliments from our customers approving the new product.

From all this, you can readily understand that we are very well pleased with our experience with Creamery Package CPFF equipment.

Very truly yours,

A. G. Marcus, President,
Crown City Dairy Co.

When Crown City Dairy, Pasadena, Calif., put its new CP Full-Flo Plate Pasteurizer in operation, 106 guests—including many health officials and milk inspectors—were given unmarked samples and asked to judge which was raw milk or pasteurized milk. So similar in flavor were all samples that not a single guest made a perfect score.

This flavor protection is but one of many reasons why CP Full-Flo Plate Pasteurizing Equipment merits your consideration. Automatic in operation and control, this modern unit lifts plant operation to a new efficiency level. Start the product through . . . and in five minutes, or less, the perfectly pasteurized and cooled milk goes into the bottle. Regeneration saves three out of four B.T.U.'s required for older systems. Savings on cooling are also substantial.

Write for Bulletin E-5.

THE CREAMERY PACKAGE MFG. COMPANY

1243 WEST WASHINGTON BLVD., CHICAGO, ILLINOIS

Sales Branches in 18 Principal Cities.

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DIFCO

Isolation and Cultivation of BRUCELLA

•

Isolation of Strains

Bacto-Tryptose Agar is recommended for isolation of *Brucella* from all types of infected milk. The medium used for this purpose should be prepared with Bacto-Crystal Violet (DC-1) to inhibit development of the Gram-positive streptococci. For isolation from blood specimens preliminary enrichment of the sample in a broth prepared with Bacto-Tryptose is recommended before inoculation on plates of tryptose agar.

•

Differentiation of Types

Bacto-Tryptose Agar to which thionin or basic fuchsin have been added is recommended for differentiation of newly isolated strains of *Brucella.*

•

Mass Cultivation

Bacto-Tryptose Agar, prepared without addition of dyes or other ingredients, supports luxuriant growth of all *Brucella.* It is an excellent medium for mass cultivation of the organisms for preparation of bacterial vaccines or antigens.

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

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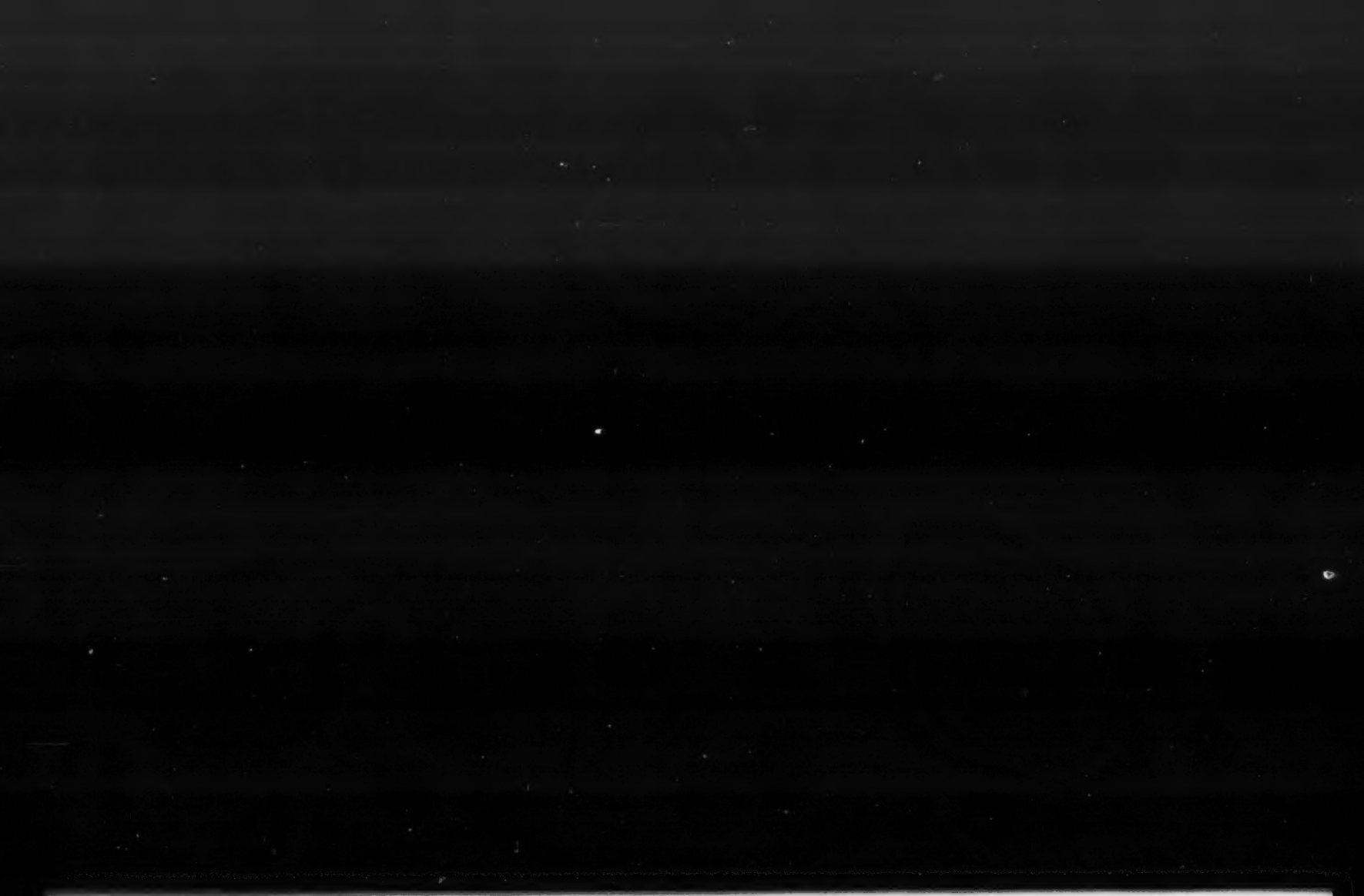
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